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Tolerance and Resistance in Wheat to Septoria Tritici Blotch and Spot Blotch

Principal Investigators: Yehoshua Anikster, Amram Eshel

Grantee Institution: Department of Plant Sciences, Tel Aviv University, Tel Aviv, Israel

Collaborators: Ram C. Sharma, Sundar M. Shrestha

Institution: Institute of Agriculture & Animal Science, Rampur, Chitwan, Nepal

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EXECUTIVE SUMMARY

There are many biotic and abiotic constraints to successful wheat production. This project was concerned with pathogens that cause severe yield losses in certain parts of the world. The main quest of the project was the attempt to combine two protection strategies, namely disease tolerance and disease resistance in the same line. The tasks were divided in to three complementing parts – identifying sources for disease tolerance and resistance, determining the mode of inheritance of these traits, and initiating a breeding program for developing new lines carrying the desired traits. While definition of disease resistance is relatively straight forward, that of disease tolerance is rather ambiguous. Disease tolerance is defined as the ability of a susceptible crop to maintain high yield even when attacked by the pathogen. Testing for tolerance involves the comparison of yield losses by susceptible tolerant and non-tolerant lines under similar disease severity. It has been found during this project that tolerance varies with disease severity. A line that was tolerant under mild epidemics broke under severe epidemic and its tolerance was lost. On the other hand, when environmental conditions limited spreading of the disease the yield losses of the non-tolerant lines were too small to compare with. Resistant lines cannot be tested for tolerance since this trait can only be shown in comparison with non-tolerant lines suffering a significant yield loss.

Selecting for disease tolerance cannot be done like selection for resistance traits. By its definition, tolerance is a trait of the line as a whole and cannot be evaluated at the individual plant level. The genetic variability of breeding products limits to a great extent the selection capabilities. We therefore tried to identify a proximate trait that could be used as a selection marker for tolerant individuals. On the basis of previous knowledge we assumed that tolerance will be correlated with performance of the photosynthetic apparatus of the individual plants. The plan was to establish a physiological test of the individual plant that could be performed in the field, which will be correlated with its level of tolerance.

The part of the project that has been carried out in Israel focused upon the pathogen *Septoria tritici*, which causes Septoria tritici blotch (STB). During the previous decade this pathogen emerged as a serious factor in wheat production in this region. Two commercial cultivars (Miriam and Nirit) were used as possible sources for STB tolerance in this program. A susceptible cultivar (Barkai) was used as a complementary susceptible line. The degree of tolerance and resistance of these parent lines, as well as that of their progenies was tested in the field. The various groups of lines tested showed variable degree of tolerance and resistance in different years of the project. The physiological tests we performed did not correlate well with the degree of tolerance in all cases. Among the products of our breeding program there are a number of promising lines that can serve as the basis for future development along these lines.

Among biotic stresses diseases are the most important in the warm areas of South Asia including the lowlands of Nepal, where this project was carried out. In the past 20 years, foliar blights have been recognized as the major disease constraint to wheat cultivation in the warmer eastern plains of South Asia. In many parts of South Asia, as is the case in the Nepal lowlands foliar blights occur as a complex of spot blotch and tan spot and is called Helminthosporium leaf blight (HLB). Earlier studies have reported that wheat genotypes adapted to the region possessed only low levels of resistance to HLB, hence subject to on average 20% wheat yield reduction on the regional basis. However, genotypes exotic to South Asia had been reported to possess high level of resistance to foliar blights in the other part of the world. This project was initiated to identify and incorporate resistance and tolerance to HLB in the local susceptible commercial cultivars of wheat, understand the mechanism of disease induced grain yield losses at different levels in the cultivars, and combine genetic resistance and tolerance to HLB in the locally adapted wheat genotypes.

RESEARCH OBJECTIVES

The goal of this project was to fill a gap in protection of wheat against HLB in Nepal and STB in Israel, where these leaf pathogens may cause loss of 20-30% in yield (4, 13).

Commercial wheat varieties grown in Nepal do not posses HLB resistance. Search for local resistance sources was vital. In Israel, Septoria tritici blotch is widespread. Variability in pathogen virulence and in RFLP markers was found to be very large compared to other wheat growing countries (5, 7). In this situation specific resistance of known sources may break down (2). As planned, the project's aim was to build a defense system in which specific resistance is backed-up by disease tolerance in order to achieve a stable lasting protection. The advantages of tolerance over resistance were discussed by several researchers (8, 11, 14, 16, 18). Although tolerance to fungal diseases, including STB has been found in other studies (1, 9, 6, 12, 15, 17), so far disease tolerance has not been incorporated as a breeding strategy due to lack of tools for tolerance estimation or tools for selection of tolerance, as well as lack of understanding the inheritance of this trait (3, 10). Another reason was the lack of understanding of the physiologic mechanism that enables compensation for loss of green leaf tissue, caused by disease stress (19).

During the research period we found out that tolerance study is very complicated on its own. thus we decided to concentrate on developing tools for tolerance measuring and in breeding for tolerance, disregarding specific resistance.

The innovative aspects of the project were:

1. Parameters for STB tolerance were developed.

2. Crosses were performed to transfer tolerance from tolerant varieties into commercial non-tolerant cultivars.

The "Hazera" Seed Company provided all facilities for the field experiments during the four years.

METHODS AND RESULTS

A. Israel

1. Breeding

One aim of this part of the project was to study the heritability of tolerance to STB (Septoria tritici blotch) using breeding techniques. Shafir (SON64/TZPP//NAI60/3/FA, Hazera Seed Co., Israel) was a common commercial cultivar. It was found to be highly susceptible to STB in agricultural fields as well as in previous studies and it showed high yield loss under severe epidemics. In this study we intended to increase the tolerance of this cultivar to STB by crossing with two tolerant sources: Miriam and Nirit. The susceptible cultivar Miriam (Capingo 53// N10/Bvr/3/Yq54/2 Merav, Volcani Center, Israel) exhibited tolerance to STB in previous studies (19, 20, 21). The yield of this cultivar did not change significantly under STB epidemics and other stresses. Nirit (F_2 CIMMYT//Bet Hashita/Degenit, Weizmann Institute, Israel), another STB susceptible, is characterized by a short grain filling period and high kernel weight under disease stress.

During the study period (1999-2003), we crossed these three cultivars, and produced the following three families: Shafir x Miriam, Shafir x Nirit and Miriam x Nirit. The crosses between Miriam and Nirit were made in order to produce an enhanced source of tolerance, by combining tolerance factors or genes. For each family, two reciprocal F1 crosses were prepared in order to study mother effect. In order to enhance tolerance, we prepared backcrosses with the tolerant parent (*e.g.* Miriam/Shafir//Miriam) or by using tolerant cytoplasm (*e.g.* Miriam//Shafir/Miriam). All crosses were prepared in the Institute for Cereal Crops Improvement, Tel-Aviv University.

For investigation of tolerance under STB epidemic, the progenies and their parents were tested in field experiments. Table 1 presents all the crosses and progenies of individual selections that were tested in the field experiments during 2001, 2002 and 2003. A list of F1 and BC seeds available for this study is presented in Table 2. Seed of all crosses and experiment-line progeny are kept at the Institute for Cereal Crops Improvement, Tel-Aviv University.

Table 1. Crosses and selections that were made in this project and harvest years in which they were tested for tolerance.

Sel 01, 02 – Selection of a BC from harvest year 2001 or 2002, respectively (first generation selection).

Sel 02-01 – Selection of a selection from nursery 2002 (second generation selection).

Family	Cross	Harvest year		
		2001	2002	2003
SHAFIR X MIRIAM	**F1:**			
	Shafir/Miriam	+	+	
	Miriam/Shafir	+	+	
	BC:			
	Miriam// Miriam/Shafir	+	+	+
	Miriam// Miriam/Shafir sel 01		+	
	Miriam// Miriam/Shafir sel 02-01			+
	Miriam// Miriam/Shafir sel 02			+
	Miriam// Shafir/Miriam		+	+
	Miriam// Shafir/Miriam sel 02			+
	Miriam/Shafir //Miriam	+	+	+
	Miriam/Shafir //Miriam sel 01		+	
	Miriam/Shafir //Miriam sel 02			+
	Miriam/Shafir //Miriam sel 02-01			+
SHAFIR X NIRIT	**F1:**			
	Shafir/Nirit	+	+	
	Nirit/Shafir		+	
	BC:			
	Nirit/Shafir //Nirit	+		
	Nirit/Shafir //Nirit sel 01		+	+
	Nirit/Shafir //Nirit sel 02-01			+
	Shafir/Nirit //Nirit		+	+
	Shafir/Nirit //Nirit sel 02			+

Table 1. Continued

Family	Cross	Harvest year		
		2001	2002	2003
MIRIAM X NIRIT	**F1:**			
	Miriam/Nirit		+	
	Nirit/Miriam	+		
	BC:			
	Miriam// Nirit/Miriam	+	+	+
	Miriam// Nirit/Miriam sel 02-01			+
	Miriam// Nirit/Miriam sel 01		+	+
	Miriam// Nirit/Miriam sel 02			+
	Nirit/Miriam //Miriam	+	+	+
	Nirit/Miriam //Miriam sel 02-01			+
	Nirit/Miriam //Miriam sel 01		+	+
	Nirit/Miriam //Miriam sel 02			+
	Nirit// Nirit/Miriam	+	+	+
	Nirit// Nirit/Miriam sel 01		+	
	Nirit// Nirit/Miriam sel 02-01			+
	Nirit// Nirit/Miriam sel 02			+
	Nirit/Miriam //Nirit	+	+	+
	Nirit/Miriam //Nirit sel 01		+	
	Nirit/Miriam //Nirit sel 02-01			+
	Nirit/Miriam //Nirit sel 02			+
	Miriam/Nirit //Miriam		+	+
	Miriam/Nirit //Miriam sel 02			+
	Miriam/Nirit //Nirit		+	+
	Miriam/Nirit //Nirit sel 02			+
	Miriam// Miriam/Nirit		+	+
	Miriam// Miriam/Nirit sel 02			+

Table 2. Crosses and amount of seeds prepared in this study during 1999-2002.

Type of cross	Cross	No. of seeds prepared	No. of seeds reserved
F1	Shafir / Miriam	651	400
	Miriam / Shafir	267	21
	Shafir / Nirit	474	227
	Nirit / Shafir	158	24
	Miriam / Nirit	191	57
	Nirit / Miriam	199	0
BC	Shafir // Shafir / Miriam	79	79
	Miriam // Shafir / Miriam	258	104
	Shafir / Miriam // Shafir	11	11
	Shafir / Miriam // Miriam	433	433
	Shafir // Miriam / Shafir	105	105
	Miriam // Miriam / Shafir	382	102
	Miriam / Shafir // Shafir	24	13
	Miriam / Shafir // Miriam	703	423
	Nirit // Shafir / Nirit	156	129
	Shafir / Nirit // Shafir	18	0
	Shafir / Nirit // Nirit	535	375
	Nirit // Nirit / Shafir	264	264
	Nirit / Shafir // Shafir	57	57
	Nirit / Shafir // Nirit	327	223
	Miriam // Miriam / Nirit	328	168
	Nirit // Miriam / Nirit	86	86
	Miriam / Nirit // Miriam	182	21
	Miriam / Nirit // Nirit	294	134
	Miriam // Nirit / Miriam	271	3
	Nirit // Nirit / Miriam	252	0
	Nirit / Miriam // Miriam	699	419
	Nirit / Miriam // Nirit	294	16

2. Summary of field experiments

Field experiments were carried out annually 2000-2003 in order to evaluate the level of tolerance according to yield losses and physiological response of the parents and crosses to STB epidemic. In 2001 and 2002 we analyzed F1 crosses and BC of the three families: Shafir x Miriam, Shafir x Nirit and Miriam x Nirit. In addition, we tested several cultivars from Nepal which were supplied to us by the cooperating investigator Dr. Ram C. Sharma. These Nepalese lines were found to be either susceptible or tolerant to HLB in Nepal. In 2002 and 2003 first and second generation selections from backcross lines studied in previous years were included in the experiments. For experimental details see annual reports.

Due to differences in the weather among the three harvest years there were differences in epidemic severity and yield losses. Even though we used supplemental irrigation during dry periods, temperature and relative humidity patterns differed markedly and so did pathogen development.

a. STB epidemic

Average AUDPC of STB for the three parents Shafir, Miriam and Nirit was significantly higher in 2003 than in previous years (Table 3). In 2001, average AUDPC of Shafir was higher than Miriam and Nirit. In 2002 all three parents were equally susceptible, and in 2003 the average AUDPC of Shafir and Nirit was higher than Miriam. In summary, among the three parents, Shafir was the most susceptible and Miriam was slightly less susceptible to STB.

Table 3. Area under disease progress curve after 109 days post inoculation of the three parents Shafir, Nirit and Miriam in three harvest years of field experiments.

Cultivar	**2001**	**2002**	**2003**	**Avg./ Cultivar**
SHAFIR	3966.0[1] a ↓	3670.4 a ↓	4207.0 a ↓	*3947 a* ↓
NIRIT	3299.6 b ↓	3273.5 a ↓	4192.7 a ↓	*3588 b* ↓
MIRIAM	3201.2 b ↓	3357.5 a ↓	3456.9 b ↓	*3338 c* ↓
Avg. /Year	*3488 b*→	*3433 b*→	*3952 a*→	

[1] Cultivar means within columns (arrow downwards) or year means within row (arrow to the right) followed by the same letter do not differ significantly ($P<0.05$) as determined by Tukey's aposteriori test.

b. Yield loss

Yield losses due to STB epidemic in 2001 were small in all three parents (Table 4) and in the crosses between them (data given in annual report) comparing with the following years. Yield losses of Miriam and Nirit were not significant in 2001. In 2002 and 2003, all the parents and almost all the crosses showed significant yield losses. Losses in 2003 were much higher than in previous years. Despite year differences, the yield losses of Miriam and Nirit were smaller than Shafir in both years. In 2001, the average yield losses of the Shafir x Nirit family were smaller than the other two families. In 2002 the averages were similar, although slightly smaller values of the Miriam x Nirit family. In 2003 all three families had similar averages of yield losses.

These data suggest that tolerance is difficult to detect in years of high epidemic. In field experiments, tolerance can be determined only relatively to a known non-tolerant cultivar.

Table 4. Percent yield losses of the three parents and family averages in the three harvest years of field experiments. Yield losses are expressed as harvest index (HI) and thousand kernel weight (TKW) of single tillers, and thousand kernel weight of a bulk of seed harvest (TKW50).

Harvest year	Yield parameter	Shafir	Miriam	Nirit	Family: Shafir x Miriam	Family: Shafir x Nirit	Family: Miriam x Nirit
2001	HI	31*	14 ns	4 ns	15	2	11
	TKW	27*	7 ns	2 ns	9	-2	7
	TKW50	15*	4 ns	-4 ns	6	-1	3
2002	HI	27*	18*	17*	23	19	17
	TKW	28*	21*	17*	22	20	16
	TKW50	31*	23*	20*	25	19	18
2003	HI	77*	66*	63*	70	73	68
	TKW	50*	26*	34*	30	37	36
	TKW50	53*	28*	35*	40	35	41

*/ ns - Significant/not significant loss in comparison with the protected plot, according to Student's t-test (P<0.05).

c. Tolerance of parents, crosses and selections

Among the three parents, Shafir as the susceptible - non tolerant cultivar had the highest yield losses in all three years, as expected. Miriam and Nirit did not have significant yield losses in 2001. In 2002 and 2003 their yield losses were smaller than that of Shafir, and smaller comparing to the average yield loss of their crosses with Shafir (Table 4), and thus, seem to behave as tolerant to STB.

Evaluation of tolerance was made for each cross and its selections based on the three harvest years of field experiments. In 2001, crosses that showed no significant yield loss were considered as tolerant. In 2002 and 2003, since most of the losses were significant, crosses with yield loss that was smaller than the average for the family were considered as tolerant. First-generation selections are plants that were selected from back-crosses for having high AUDPC and high yield comparing to the other plants from the same cross. These selections were made in the 2001 and 2002 nurseries, and their tolerance was evaluated again in 2002 and 2003 nurseries, respectively. Second-generation selections were selected in the same way from the first-generation, and were evaluated again in the 2003 nursery.

Table 5 summarizes the response of each cross and its selections throughout the three harvest years of field experiments, 2001-2003.

F1 crosses between Shafir and Miriam showed tolerance in both ways (Shafir/Miriam and Miriam/Shafir) in the two test years. Among the back-crosses in this family, only selections of Miriam/Shafir//Miriam were tolerant in the first and second generation.

The F1 cross Shafir/Nirit was tolerant in the two test years, but its reciprocal cross Nirit/Shafir, was not tolerant. The backcross Nirit/Shafir//Nirit and its first generation selection were tolerant, but the second generation selection was only moderately tolerant.

The F1 cross Nirit/Miriam was tolerant in 2001, but the reciprocal cross Miriam/Nirit was not tolerant in 2002. The most tolerant among the backcrosses of this family was Nirit/Miriam//Nirit, which was tolerant in 2001 (although not in 2002 and 2003), as well as its first and second generation selections.

In summary, some F1 and BC descendants were not tolerant, even in the family Miriam x Nirit. Selections of relatively tolerant plants did not necessarily maintain their tolerance in the first and the second generations.

Selections that exhibited relative tolerance were:

BC: Nir/Shf//Nir – Selection #80 from 2001 was tolerant and second generation selection #312 from 2002 was moderately tolerant.

BC: Nir/Mir//Nir - Selection #105 from 2001 was tolerant and second generation selections #321 and #323 from 2002 were moderately tolerant.

Table 5. Evaluation of tolerance of crosses and selections grown in field experiments during 2001, 2002 and 2003 at Mivhor Farm, Israel.

Each table presents crosses from the same family ("Cross"), their response at the field experiments ("General response"), the response of the first generation selection from 2001 or 2002 ("1st gen. selection") and the response of the second generation selection ("2nd gen. selection").

Abbreviations:
Cultivars: Shf = Shafir; Mir = Miriam; Nir = Nirit
Type of response: T = tolerant; M = moderately tolerant; NT = not tolerant
Nursery harvest years: '01, '02, '03 = 2001, 2002 and 2003 respectively
Number of selected plants is in brackets

A. SHAFIR X MIRIAM

Cross	General response	1st gen. selection	2nd gen. selection
F1: Mir/Shf, Shf/Mir	T in '01 and '02		
BC: Mir// Mir/Shf	NT in '01-'03	'01- M (1) '02- NT (2)	NT (2)
BC: Mir// Shf/Mir	M in '02, NT in '03	'02- NT (2)	
BC: Mir/Shf //Mir	NT in '01-'03	'01- M (1) '02- 1 plant T (2)	T (1)

B. SHAFIR X NIRIT

Cross	General response	1st gen. selection	2nd gen. selection
F1: Shf/Nir	T in '01 and '02		
F1: Nir/Shf	NT in '02		
BC: Nir/Shf //Nir	T in '01	'01- T (1)	1 plant M (2)
BC: Shf/Nir //Nir	M in '02, NT in '03	'02- NT (2)	

C. MIRIAM X NIRIT

Cross	General response	1st gen. selection	2nd gen. selection
F1: Mir/Nir	NT in '02		
F1: Nir/Mir	T in '01		
BC: Mir// Nir/Mir	NT in '01 and '02, M in 03	'01- T (1) '02- NT (2)	NT (1)
BC: Nir/Mir //Mir	NT in '01-'03	'01- NT (1) '02- 2 plants M (3)	NT (3)
BC: Nir// Nir/Mir	NT in '01-'03	'01- T (1) '02- 1 plant T (2)	NT (1)
BC: Nir/Mir //Nir	T in '01, NT in '02-'03	'01- T (1) '02- NT (2)	2 plants T (3)
BC: Mir/Nir //Mir	T in '02-03	'02- NT (2)	
BC: Mir/Nir //Nir	NT in '02-03	'02- NT (2)	
BC: Mir// Mir/Nir	NT in '02-03	'02- NT (1)	

d. Photosynthesis

Another of the aims of this project was to study the photosynthetic capabilities of infected and non-infected plants of susceptible cultivars in order to identify possible mechanism of STB tolerance, and to establish a method for identifying tolerant individuals. The nature of the disease is that islands of green tissue remain active among the damaged parts of the tissue. The underlying assumption is that up-regulation of photosynthesis in these parts of the leaves compensate for the loss of green area due to necrosis and pycnidial coverage. We have therefore measured photosynthetic rates of leaves of infected and non-infected plants grown in the field and in the net house.

A synopsis of the data collected for 42 breeding lines is presented in Figure 1. The tested lines included the parents – Miriam, Nirit, and Shafir – F1's and selected backcrosses. For every line ratios of photosynthesis performance of the infected over the protected lines was calculated for two types of data, rate of photosynthesis calculated per unit chlorophyll and the rate of photosynthesis calculated per unit green leaf area. Ratios above the unit line indicate enhancement while those below the line indicate inhibition. It can be seen that in more cases enhancement of the rate of photosynthesis per unit chlorophyll was observed than for the rates calculated per unit green leaf area. The data were plotted against AUDPC values determined for these lines. Weak, though non-significant correlations can be observed. Similar attempts to find correlations between these parameters of photosynthetic performance and yield losses did not indicate any significant values.

It can be concluded that indeed enhancement of the rate of photosynthesis per unit chlorophyll content is a possible mechanism for STB tolerance. The negative correlations may indicate that this mechanism may be of value at low epidemics, but not under severe ones. However, other parameters may affect the photosynthetic performance and therefore the correlations between photosynthetic performance and yield losses are too weak to be of value for identification of tolerant individual plants.

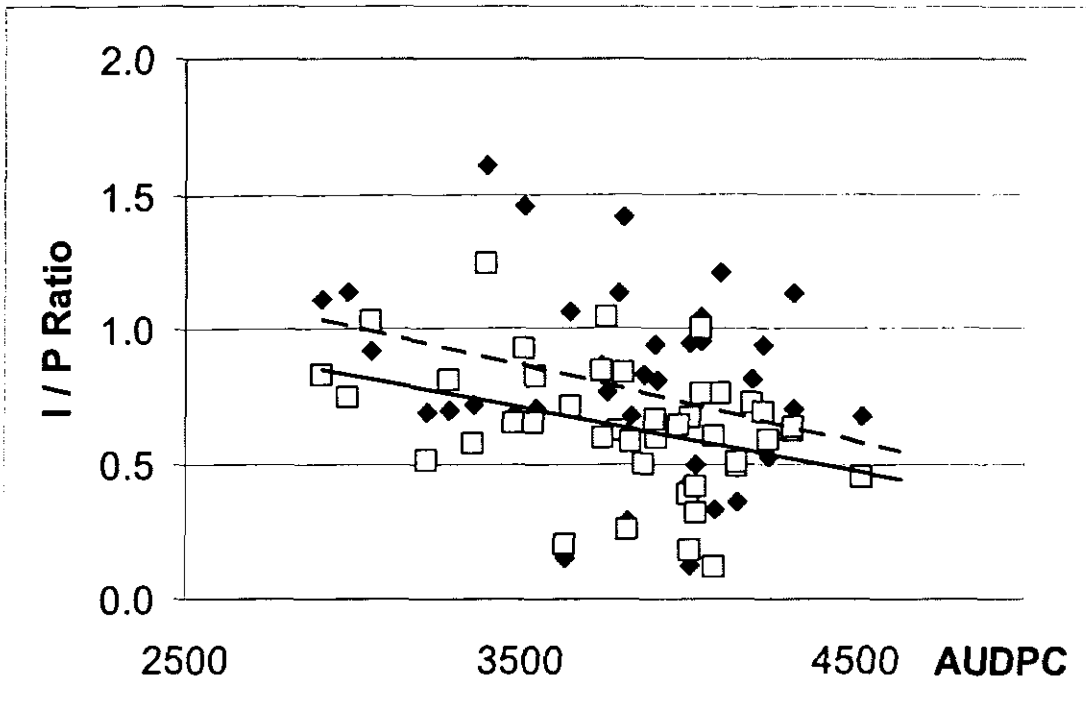

Figure 1. Ratio of photosynthetic rates (Calculated per unit chlorophyll – closed diamonds, dashed line or calculated per unit leaf area – open squares, solid line) of infected over protected wheat plants of 42 breeding lines (Parents, F1's and Selected backcrosses) plotted against AUDPC.

e. The response of cultivars from Nepal to STB in Israel

Susceptibility and tolerance to STB of the Nepalese cultivars: UP262, Nepal 297, RR21 and HD2329 were evaluated in the field experiments of 2001 and 2002. All the cultivars were moderate to highly susceptible to STB isolate ISR8036 (Table 6). The most susceptible cultivars were: UP 262 and RR21. RR21 was tolerant to STB according to the two yield parameters HI and TKW. Yield loss of this cultivar did not reduce significantly in 2001, and in 2002 the yield loss was similar to that of the tolerant Israeli cultivars Miriam and Nirit. Nepal 297 was tolerant in 2002 and partly tolerant in 2001. UP 262 was tolerant in 2001 but not in 2002 and HD2329 was the least tolerant.

UP262 and Nepal 297 were resistant and HD2329 and RR21 were susceptible to natural epidemic of yellow rust in 2001 (data presented in the annual report).

H991 ("Hazera Seed Co.", Israel) is a local susceptible cultivar, that showed relatively small yield losses under natural epidemics in variety trials in Israel. This cultivar was incorporated in the 2001 and 2002 field experiments. In both years, the AUDPC of this cultivar was the lowest among all the cultivars, from Israel and from Nepal (Table 6). Therefore, the small losses in harvest index and thousand kernel weight which this cultivar showed in these years were probably a result of its relative resistance to the *S. tritici* isolate that was applied in out experiments.

Table 6. Reaction to STB and yield parameters of four Nepalese cultivars comparing with the Israeli cultivars: Shafir, as not-tolerant, Miriam and Nirit as tolerant and H991 as moderately susceptible. Field experiments at Mivhor Farm, Israel.

A. Season 2001

Origin	Cross/Cultivar	STB reaction (AUDPC)		HI[1] protected	% loss	TKW[2] protected	% loss
Israel	SHAFIR	2280[3]	a	0.81	30.6*	31.58	26.8*
	MIRIAM	1673	bc	0.77	14.3	35.87	6.6
	NIRIT	1819	b	0.80	4.4	36.91	2.5
	H 991	1401	c	0.72	13.7	36.74	-1.0
Nepal	UP 262	2612	a	0.61	-6.9	38.02	1.8
	NEPAL 297	1849	b	0.59	40.1*	31.89	14.8
	HD 2329	1761	bc	0.78	32.6*	39.12	28.9*
	RR 21	2263	a	0.51	-27.0	33.59	-30.1*

B. Season 2002

Origin	Cultivar	AUDPC		HI	% loss	TKW	% loss
Israel	SHAFIR	3670[3]	ab	1.04	27.0*	45.8	27.8*
	MIRIAM	3357	abc	1.01	18.5*	50.0	20.8*
	NIRIT	3273	abc	1.10	16.9*	45.1	17.2*
	H 991	1856	d	0.92	11.0	54.0	8.9
Nepal	UP 262	3831	a	0.91	19.1*	56.1	25.9*
	Nepal 297	3087	c	0.94	7.9*	56.4	8.7*
	HD 2329	3024	c	1.05	25.6*	48.3	14.1
	RR 21	3213	bc	0.99	17.8*	58.5	14.2*

1, 2 Harvest index and thousand kernel weight of the protected plants and percent yield loss of the inoculated plants, respectively.

3 Cultivar means within columns (arrow downwards) or year means within row (arrow to the right) followed by the same letter do not differ significantly (P<0.05) as determined by Tukey's aposteriori test.

a,b,c Different letters indicate *significant difference by Tukey's aposteriori test* (P<0.05).

* Significant yield loss by Student's t-Test (P<0.05).

B. Nepal

Methods and results are included here briefly. Detailed presentation is included in Appendixes 1-8.

Methods

All wheat genotypes reported in the regional literatures being used as a source of HLB resistance were tested for their level of resistance in two seasons each in a farmer's field and at a research station. To precisely assess the loss due to disease, half of each plot was protected by multiple sprays of a fungicide while other half was exposed to natural inoculum pressure of the pathogens. Disease and agronomic characters as well as grain yield losses due to disease were investigated. Disease resistant exotic wheat genotypes and susceptible commercial cultivars were crossed to produce hybrid progenies, which were further studied to determine the genetic control of resistance to HLB. Several crosses were studied by using a novel selection index to determine the possibility of simultaneously improving disease resistance, early maturity, and high kernel weight, the three important characters for a high yielding cultivar to be successful in the region. As a result of above simultaneous selection correlated response in grain yield was investigated. The superior genotypes developed through the above procedure were further evaluated in farmer's field to determine how suitable the improved genotypes were on the bases of farmers' preference.

To determine distribution and dominance of the pathogens causing HLB, farmers' field surveys were conducted covering over 1000 km east-west high way across the plains of Nepal in each of the four years (1997-2000). Diseased leaf sample were collected and presence of pathogens and their frequency was determined in the lab using appropriate methods of detection.

Results

1. Source of Resistance

In a set of 60 wheat genotypes of diverse origins, we found that late maturity, higher disease resistance, and low to high grain yield and kernel weight characterized genotypes exotic to South Asia. However, many commercial cultivars adapted to the region, despite showing either susceptibility or low resistance to disease, were early to intermediate in maturity and had high grain yield and kernel weight. Several genotypes with high levels of disease resistance and acceptable agronomic traits were identified, which underlines the potential for further improving disease resistance using local commercial wheat cultivars and selective breeding in this germplasm, with prospects of spillover effects to other warmer areas.

2. Genetic Control of Resistance

Resistance to HLB is controlled by complex genetic mechanisms depending on the source of resistance. Both qualitative and quantitative inheritance was found in specific crosses. Additive as well as non-additive gene actions controlled resistance. Resistance and maturity didn't show genetic linkage.

3. Selection index to improve resistance, early maturity and high kernel weight

The results showed that selection for early maturing, HLB-resistant wheat lines with high grain yield and kernel weight is possible using a simple selection index that included the three traits.

4. Incidence of pathogens of causing Helminthosporium leaf blight

Both *B. sorokiniana* (causing spot blotch) and *P. tritici-repentis* (causing tan spot) pathogens were found on wheat crop grown across Nepal lowlands in all four years (1998 to 2001) and their incidence could change unpredictably. Hence, a continuous monitoring of these pathogens is necessary in order to manage them effectively. Wheat breeding programs in South Asia need to incorporate resistance genes for these two pathogens, possibly from distinct pools, in order to develop cultivars with durable resistance to HLB.

IMPACT RELEVANCE AND TECHNOLOGY TRANSFER

The project helped strengthen manpower and research facilities at the institute and within Nepal. The research activities help initiate collaboration with national and international wheat research institute. Information disseminated through the publication of journal articles and conferences paper would be valuable for wheat researchers in South Asia and other warm areas of the world.

The distribution of the two pathogens causing leaf blight in wheat in the lowlands is better understood now. The information in the shift in the predominance of the two pathogens would be directly helpful to the wheat breeders in planning incorporation of additional resistance genes and their geographical deployment.

Epidemiology of HLB causing pathogens is better understood. We have first time documented that tan spot could become a problem in warm areas in South Asia, where spot blotch used be the major disease. Also, we have documented new ways to assess foliar blight damage. Disease severity per day or per degree day was better correlated with grain yield losses than area under disease progress curve. Also, we have explicitly documented that late seeding of wheat suffer a much greater loss from foliar blight compared to timely seeded wheat and explained why is so. These findings underline the important effect of various environmental factors on HLB development and could lead to new guidelines for improving control of these effects as part of an integrated crop management strategy.

Information on the complex genetics of leaf blight in many wheat varieties resistant to HLB is largely understood. This information is expected to assist wheat breeders in developing selection strategies early in the breeding program.

A simple selection index to simultaneously select for HLB resistance, early maturity, and heavier kernels in wheat was successfully tested. It is expected that wheat breeders in warm wheat growing areas would use this selection technique. This selection technique has been published in Crop Science (2003), 43:2031-2036.

Several wheat lines have been developed that possess background tolerance of adapted local wheat varieties and high level of resistance to HLB from exotic wheat genotypes. These lines were tested in farmers' fields and a few of them were liked by the farmers on their preference criteria.

Research facilities at IAAS were strengthened through the project funds, which greatly helped in achieving the project activities as well other research activities.

Three faculty members at IAAS and seven postgraduate students were directly benefited by having an opportunity to learn research techniques within the scope of this project. Six of these students are already working in different agencies in Nepal. Thus the project helped in manpower development and transferring technology from an academic institution to other service institutions.

The project activities created awareness among students and scientists as well as external collaborators to initiate further research on foliar blight in Nepal involving other scientists. Two such institutions are CIMMYT, Katmandu, Nepal and Cornell University, USA.

Further research on foliar blights is ongoing at this institute and in Nepal focusing more on climatic effect on diseases severity and yield losses. Ongoing and future research has a thrust on understanding the dynamics of this disease in the resource poor farmers' fields.

PROJECT ACTIVITIES/OUTPUTS

A. Publications

1. Sharma, R.C., and E. Duveiller. 2003. Selection index for improving Helminthosporium leaf blight resistance, maturity, and kernel weight in spring wheat. In: Press Crop Science 43:2031-2036.
2. Sharma, R.C., and M.R. Bhatta, 1999. Inheritance of field resistance to spot blotch in three wheat crosses. J. Inst. Agric. Anim. Sci. 19-20: 111-118.
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4. Sharma, R.C., and E. Duveiller. 2003. Improving the effectiveness of selection for resistance to Helminthosporium leaf blight in wheat. p. 51-54. In: J.B. Rasmussen, T.L. Friesen, and S. Ali (ed.) Proc. of the 4th Int. Wheat Tan Spot and Spot Blotch Workshop, Bemidji. 21-24 July. North Dakota State University, Fargo, USA.
5. Sharma, R.C., S, N, Sah, S. Gyawali. and E. Duveiller. 2003. Genetic control of resistance to Helminthosporium leaf blight in wheat. P. 68-73. In: J.B. Rasmussen, T.L. Friesen, and S. Ali (ed.) Proc. of the 4th Int. Wheat Tan Spot and Spot Blotch Workshop, Bemidji. 21-24 July. North Dakota State University, Fargo, USA.
6. Sharma, R.C., S.M. Shrestha, and E. Duveiller. 2003. Incidence of *Bipolaris sorokiniana* and *Pyrenophora tritici-repentis* on wheat in the lowlands of Nepal. P. 122-127. In: J.B. Rasmussen, T.L. Friesen, and S. Ali (ed.) Proc. of the 4th Int. Wheat Tan Spot and Spot Blotch Workshop, Bemidji. 21-24 July. North Dakota State University, Fargo, USA.
7. Sharma, R.C., and E. Duveiller. 2003. Effect of stress on Helminthosporium leaf blight in wheat. P. 140-144. In: J.B. Rasmussen, T.L. Friesen, and S. Ali (ed.) Proc. of the 4th Int.

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8. Sharma, R.C., S. Gyawali, S.M. Shrestha, N.K. Chaudhary, and E. Duveiller. 2003. Field resistance to Helminthosporium leaf blight in wheat genotypes from diverse origins. P. 145-150. In: J.B. Rasmussen, T.L. Friesen, and S. Ali (ed.) Proc. of the 4th Int. Wheat Tan Spot and Spot Blotch Workshop, Bemidji. 21-24 July. North Dakota State University, Fargo, USA.

9. Sharma, R.C., Y. R. Kandel, E. Duveiller, and S.M. Shrestha. 2003. Characterization of Helminthosporium leaf blight resistance in wheat at different growth stages. Pp. 153-158. In: J.B. Rasmussen, T.L. Friesen, and S. Ali (ed.) Proc. of the 4th Int. Wheat Tan Spot and Spot Blotch Workshop, Bemidji. 21-24 July. North Dakota State University, Fargo, USA.

B. Thesis research completed with partial funding through the project

1. Helminthosporium Leaf Blight of Wheat: Genetic and Management Studies – Sanjaya Gyawali (2000).
2. Diallel Analysis of Wheat Genotypes for Resistance to Helminthosporium Leaf Blight – Surya N. Sah (2000).
3. Physio-Morphological Traits Associated with Resistance and Tolerance to Helminthosporium Leaf Blight in Spring Wheat - Umesh Rosyara (2002).
4. Effect of Nitrogen, Phosphorus, Potash, and Chlorine on Helminthosporium Leaf Blight and Performance of Wheat – Pramod Sharma (2002).
5. Incidence of *Bipoloaris sorokiniana* and *Pyrenophora tritici-repentis* and Characterization of Host Resistance in Wheat – Yuba Raj Kandel (2003).
6. Effect of Tillage and Weed Flora on Performance of Wheat Genotypes and the Severity of Helminthosporium Leaf Blight – Vijaya Bijukachhe (2003).
7. Helminthosporium Leaf Blight of Wheat: Inheritance and Association of Traits – Bhai Raja Pandey (2003).
8. Photosynthesis as a Tolerance Mechanism of Septoria tritici Blotch in Wheat – Hani Sarfati (2001).
9. The Mutual Relations between Isolates of *Septoria tritici* Varying in Virulence on Several Wheat Genotypes – Smadar Ezrati (2003).

C. Conferences attended

1. Fifth International Septoria Workshop, 20-24 September, 1999, CIMMYT, Mexico.
2. Third International Crop Science Congress, 17-23 August, 2000, Hamburg, Germany.
3. Fourth International Wheat Tan Spot and Spot Blotch Workshop, 21-24 July, 2002 Bemidji, USA.

PROJECT PRODUCTIVITY

A. Israel

The screening of plant genotypes for high degree of tolerance to a plant pathogen is in conflict with the search of pathogen resistance by the same plant types. Resistance is manifested by reduced proliferation of the pathogen and small amount of symptoms. Tolerance, on the other hand, can only be manifested by sensitive plants that exhibit a high degree of infection. If the yield losses sustained by these infected plants are small, in comparison with non-tolerant sensitive lines, they are regarded tolerant. Therefore, those genotypes that exhibit resistance cannot show tolerance. This inherent problem is especially acute when Septoria tritici blotch is studied. There is no clear cut between sensitive and resistant response to this pathogen. The development of the disease symptoms on infected plants is gradual and even resistant plants may show up to 20% loss of green leaf area.

By the analysis of the project's preliminary results, we found that tolerance could not be investigated if it is combined with resistance because of the masking effect (as yield losses could not be measured under low disease severity). Considering this conclusion, most efforts and time were held according to the following guidelines:

1. Clear parameters were established for a cultivar to be considered as tolerant. Since tolerance is defined by small or no yield loss under disease stress, the primary condition for detecting tolerance was high disease severity (represented by AUDPC). Yield was measured by harvest index (seed weight/plant weight) and by thousand kernel weight. Yield loss was calculated by comparing the yield of diseased plants (artificially inoculated) to that of healthy plants (chemically protected). Yield loss of crosses and selected lines was compared with yield loss of the parents.

2. Experiments were carried out in the field using controlled artificial inoculation in natural climatic conditions suitable for both, the host and the pathogen.

3. For the comparison of tolerant and non tolerant cultivars, and for a better understanding of the genetics and the inheritance of the tolerance character, crosses and back-crosses between tolerant and susceptible cultivars were carried out. All offspring were studied in field nurseries and were compared to the tolerant cultivars.

According to these guidelines, we accomplished the preparation of crosses and back-crosses, including selected lines, developed parameters for identifying tolerance and held field experiments every year of the project's time period.

B. Nepal

Prior to start of the project, there was limited documentation on characterization of host resistance and pathogen variability for Helminthosporium Leaf Blight of wheat in the region. The project was successful in achieving its objectives and providing information gap to a large extent. The range of host resistance for resistance to Helminthosporium leaf blight for the wheat genotypes in the region are more or less established now.

FUTURE WORK

This project produced a large amount of germplasm, including seed of F1 generation, back-crosses and selected back-cross lines, which are ready for continuation of the research in any country. According to the findings of this project, further investigation of tolerance should be held using this germplasm. The research should be carried out over several seasons (years) in order to eliminate environmental effect on the expression of tolerance. In addition to that, the experiments should include replications of each plot, in order to evaluate statistically the yield loss of the offspring in comparison to the parents. A special focus should be made on selected lines, which genetically are more uniform.

Further research works on Helminthosporium leaf blight are continuing at the institute capitalizing on the information and facilities generated through the project. On-going works are particularly emphasizing understanding of climatic and management factors on the stability of host resistance and managing the disease to reduce yield losses. Students who were trained in this project are undertaking further research on this disease at other research institutions as well. Funding from within Nepal as well as from external sources are being made available for further research on foliar blights of wheat in Nepal.

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Appendix 1. Field resistance to Helminthosporium leaf blight in wheat from diverse origins.

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Helminthosporium leaf blight (HLB) is a serious wheat disease of wheat in South Asia, in particular in the lowlands of Nepal.. Since the leading wheat cultivars are either susceptible to HLB or possess low levels of resistance, a great deal of efforts has taken place in Nepal and in the region to identify HLB-resistant wheat. A study was conducted to determine the level of resistance to HLB and other desirable characters in a set of 60 wheat genotypes from diverse genetic background and origins. This information is needed to determine the range of host resistance available and identify resistant and/or tolerant wheat that might also possess acceptable agronomic characters to be used in the breeding program

Methodology

The set of 60 wheat genotypes from diverse genetic backgrounds and origins included in the study is given in Table 1. These materials have different yield potentials and were identified for their HLB resistance in other environments. The list includes many exotic wheat genotypes that had been specifically recycled in wheat breeding programs in South Asia for the purpose of improving resistance to HLB. Several commercial wheat cultivars from South Asia were also integrated to the study.

All genotypes were field evaluated under natural inoculum pressure, in a Randomized Complete Block with four replications during two winter seasons in 1998-1999 (1999) and 1999-2000 (2000) at two sites in Nepal (Manara and Rampur) where HLB is a severe problem every year. In each replication, plots were arranged in strips corresponding to fungicide sprayed and non-protected plots. Protected plots were sprayed four times with Tilt ^{TM} (a.i. propiconazole) at 125 g a.i./ha.. Both trials were timely sown using120-kg/ha seed. Fertlizers were applied at the rate of 120, 60, and 40-kg/ha of N P_2O_5 and K_2O, respectively. Other management practices were consistent with the good crop husbandry recommended in the region..

Twenty primary tillers in each plot were randomly selected and tagged to score HLB severity. Disease incidence was recorded after anthesis when necrosis of the penultimate leaves started. Disease severity was rated by visually assessing the percent diseased leaf area on flag (F) and F-1 leaves. For differences in disease severity are reported to be small, in four disease scores were taken to calculate the area under disease progress curve (AUDPC) and better estimate small changes in disease development during the cropping season. The collected agronomic data included days to heading (GH), plant height (cm), effective number of tillers/m^2, biomass, grain yield (GY) and thousand-kernel weight (TKW). An analysis of variance was used to determine genotypic differences and genotype-by-environment (location and year) interactions.

Losses in GY and TKW were estimated to corroborate the presence of genetic tolerance for HLB and assess yield potential or adaptation. High yielding genotypes under the conditions of this study and showing a relatively high AUDPC but with low reduction in TKW and GY were considered tolerant to HLB.

Results

The 60 genotypes significantly varied in AUDPC with mean values across sites and years ranging between 45 and 1268 suggesting a wide range of HLB resistance (Table 1). A few genotypes were highly resistant: SW 89-5193, SW 89-3060', SW 89-5422, Chirya 7, Ning 8319, and Croc 1/*Ae.sq.*//Borl95. The materials originated from China or included the infusion of alien wheat relatives. In general, these highly resistant genotypes had lower biomass, low to intermediate grain yield, harvest index and TKW. Moreover, in growing conditions of the Gangetic plains these genotypes were heading late compared to commercial cultivars of Nepal. A few locally adapted but not recently released cultivars showed the highest disease susceptibility (AUDPC): RR 21 (Sonalika), BL 1135, BL 1022, Lumbini and Siddhartha. These genotypes are usually heading early and have low to high grain yield and biomass, and intermediate to high harvest index and TKW. These results suggest that it could be possible to select wheat genotypes with low AUDPC, and acceptable levels of GY and TKW among entries not directly adapted to South Asia such as SW89-5422, Chirya 3, Ning 8319, NL785 which could further be used in crosses with current commercial genotypes to improve HLB resistance.

The reductions in GY and TKW due to HLB ranged from 2.2 to 25.6%, and 2.6 to 33.0%, respectively (Table 2). Correlation coefficients (r) for AUDPC with loss in GY (r=0.644**) and TKW (r=0.664**) were highly significant confirming that HLB overall may cause substantial economic damage should susceptible wheat cultivars be promoted. In general, reduction in GY and TKW were higher in local commercial cultivars compared to the introduced wheat genotypes. However, several adapted cultivars released in South Asia showed comparatively low reductions in grain yield and TKW. BL1473, Nepal 297, UP 262, BL1022, Kanchan and BL1813 belong to that group and showed relatively low reductions in GY and TKW despite a higher AUDPC. Based on this result and considering the lower reduction in GY and TKW, these genotypes could be considered tolerant to HLB. These materials should be recommended as parents for future crossing schemes with HLB resistant less adapted wheat genotypes to combine both resistance and tolerance to obtain possibly more durable and stable resistance to foliar blights in South Asia.

Biplot analysis identified several genotypes that were HLB-resistant and agronomically superior (Fig. 1). Results suggest it is possible to improve HLB resistance of local wheat cultivars based on selective breeding using this pool of germplasm.

Conclusion

A number of genotypes with high levels of HLB resistance and acceptable agronomic traits were identified, which underlines the potential for further improving HLB resistance using local commercial wheat cultivars and selective breeding in this germplasm, with prospects of spillover effects to other warmer areas.

Table 1. Mean values for various traits in 60 wheat genotypes evaluated for HLB resistance and agronomic values across two locations in Nepal in 1999 and 2000.

Genotype				Biomass	Grain	Harvest	Thousand	Days to	Days to	Plant	Tiller
No.	Name	Origin	AUDPC	yield	yield	index	kernel wt.	heading	maturity	height	number
				kg ha^{-1}	kg ha^{-1}	%	g			cm	m^{-2}
1.	SW 89-5193	China	45	8833	3511	39.8	37.7	89	126	69	262
2.	SW 89-3060	China	62	8961	3235	35.8	30.3	91	126	73	272
3.	SW 89-5422	China	73	9098	3428	38.3	40.9	86	123	91	205
4.	Chirya 7	CIMMYT/Nepal	77	10168	4141	40.8	37.1	84	125	97	271
5.	Ning 8319	China	79	8853	3673	41.1	40.0	84	126	96	246
6.	NL 781	CIMMYT/Nepal	81	10526	3993	37.9	39.7	88	125	93	308
7.	Croc 1/A. sq.//Borl 95	CIMMYT	81	9358	2585	28.6	35.4	91	126	89	334
8.	Chirya 3	CIMMYT/Nepal	85	10492	4429	42.3	42.2	83	125	95	272
9.	G 162		96	8788	3118	35.1	36.3	88	125	83	242
10.	Chirya 1	CIMMYT/Nepal	99	9620	3899	39.6	40.6	82	124	93	269
11.	Yangmai -6	China	101	8489	3251	36.6	41.5	86	122	107	229
12.	NL 785	CIMMYT/Nepal	108	9963	4177	41.4	41.8	83	125	97	267
13.	NL 750	CIMMYT/Nepal	112	10823	4640	42.7	41.2	82	125	97	269
14.	Sabuf	CIMMYT	113	9988	3761	37.4	40.4	83	124	99	267
15.	HLB 19		129	10249	3791	36.9	34.1	84	124	103	344
16.	NL 868	CIMMYT/Nepal	145	10539	4208	40.8	37.9	85	125	93	300
17.	BL 1740	Nepal	155	9442	3546	38.8	33.9	87	125	96	268
18.	Jinmai 4058	China	158	11128	3814	34.6	40.3	85	123	119	281
19.	Ning 8201	China	164	9123	3796	42.6	44.8	82	123	89	226
20.	K 8027	India	172	11139	3805	32.1	43.3	89	126	111	300
21.	K 7	Zambia	176	12351	4548	37.4	43.3	78	124	120	311

22. WH 542	India	180	9617	3756	38.8	29.4	83	121	88	289
23. Mayoor (HLB 48)	CIMMYT	181	10711	3232	30.3	29.0	92	126	110	328
24. PRL / Toni	CIMMYT	187	11328	3927	34.5	39.2	82	124	104	297
25. Longmai -10	China	193	13114	4318	33.0	35.5	81	121	124	302
26. ZSH 22 (Rwerere)	Zambia	236	9473	3503	36.9	39.4	77	120	118	290
27. Achyut	CIMMYT/Nepal	249	11830	4110	34.6	40.0	83	125	110	313
28. Ocepar 7	Brazil/CIMMYT	269	9672	3849	40.0	33.3	76	122	88	294
29. BH 1146	Brazil	297	11391	3690	31.6	39.4	77	121	141	330
30. BL 1813	Nepal	300	11175	4495	41.5	44.7	73	121	109	256
31. NL 872	CIMMYT/Nepal	304	9279	4002	42.0	43.0	78	122	87	250
32. NL 835	CIMMYT/Nepal	325	9511	4340	45.5	43.8	73	120	95	244
33. Triveni	CIMMYT/Nepal	332	10769	4124	38.5	42.0	73	121	105	265
34. NL 588	CIMMYT/Nepal	332	10253	4410	42.0	42.3	77	120	101	253
35. Rohini	CIMMYT/Nepal	351	10032	4064	39.5	42.0	74	121	103	249
36. BL 1692	Nepal	366	9257	4040	43.3	44.6	71	119	88	260
37. Fang 60	China	367	11507	4544	37.9	34.0	75	122	105	313
38. Bhrikuti	Nepal	368	10042	4161	41.4	39.2	76	123	91	280
39. BL 1724	Nepal	389	10343	4103	39.4	40.0	77	123	97	294
40. NL 788	CIMMYT/Nepal	389	10068	4316	42.0	42.9	78	121	98	283
41. NL 753	CIMMYT/Nepal	390	10114	4426	43.8	45.6	76	123	98	238
42. Annapurna-1	CIMMYT/Nepal	395	10064	3973	39.1	34.2	82	124	94	303
43. Vaskar	CIMMYT/Nepal	404	9616	4068	42.9	34.6	74	121	89	297
44. Nirit	Israel	417	9465	4068	42.7	37.3	72	119	90	285
45. Kavkaz/K4500	Israel	418	12462	4145	33.3	33.5	83	120	105	372
46. NL 731	CIMMYT/Nepal	435	9168	3640	40.2	44.2	73	120	104	288
47. Kanchan	Bangladesh	437	9634	3356	36.4	37.9	70	124	106	333
48. BL 1473	Nepal	444	9011	3921	43.1	48.7	67	120	103	262
49. Nepal 297	CIMMYT/Nepal	478	9136	3913	42.4	50.8	67	121	93	283
50. BL 1530	Nepal	523	10796	4181	39.3	41.5	76	121	103	266
51. Shafir	Israel	528	9079	4077	44.8	38.6	68	119	85	291

52. UP 262	India	559	11515	4553	40.6	45.9	73	120	105	302
53. Vinayak	CIMMYT/Nepal	560	10606	3961	36.7	38.8	70	119	86	339
54. Nepal 251	CIMMYT/Nepal	662	10066	4335	41.6	40.2	72	120	102	292
55. Siddhartha	CIMMYT/Nepal	799	8761	3705	42.9	38.8	68	119	83	316
56. Lumbini	CIMMYT/Nepal	885	8634	3508	39.9	42.9	69	119	91	282
57. BL 1022	Nepal	994	10338	4328	41.6	42.8	70	119	97	246
58. HD1982	India	1017	9520	3760	38.5	42.2	69	119	86	312
59. BL 1135	Nepal	1195	8673	3861	43.9	37.4	69	120	106	287
60. RR 21 (Sonalika)	CIMMYT/Nepal	1268	8174	3565	41.4	45.3	66	120	100	257
Average		346	10036	3928	39.1	39.8	78	122	98	283
$LSD_{0.05}$		73	1512	442	2.3	3.3	5	4	12	45
CV (%)		15.2	10.9	8.1	4.2	6.0	4.6	2.4	8.8	11.5

Table 2. Losses due to Helminthosporium leaf blight in biomass yield, grain yield, 1000-kernel weight, and harvest index in 60 wheat genotypes averaged in 1999 and 2000

Genotype	Biomass yield	Grain yield	1000-kernel weight	Harvest index
	----------	---------- %	----------	----------
SW 89-5193	2.0	3.4	3.9	8.6
SW 89-3060	4.3	4.3	2.6	18.2
SW 89-5422	3.8	4.2	3.5	4.9
Chirya 7	5.2	2.2	5.0	2.2
Ning 8319	6.2	5.4	4.5	4.9
NL 781	8.8	6.2	4.3	4.4
Croc 1/A. sq.//Borl 95	8.7	4.6	3.7	11.4
Chirya 3	4.3	8.4	4.8	2.2
G 162	12.6	5.9	5.1	7.5
Chirya 1	1.8	7.0	5.3	2.6
Yangmai -6	2.8	6.8	4.7	9.0
NL 785	3.8	8.7	7.0	10.0
NL 750	4.3	7.6	6.2	8.2
Sabuf	6.8	5.8	4.5	4.5
HLB 19	10.5	6.3	5.6	3.5
NL 868	9.6	6.5	15.5	5.2
BL 1740	7.2	17.6	8.7	4.3
Jinmai 4058	13.4	5.9	5.2	3.6
Ning 8201	6.8	5.7	4.5	2.4
K 8027	15.2	6.6	4.9	7.7
K 7	5.6	7.0	5.6	2.8
WH 542	16.1	10.5	5.2	11.8
Mayoor (HLB 48)	14.6	7.6	4.0	3.8
PRL / Toni	11.1	5.4	4.9	8.2
Longmai -10	4.9	6.4	5.8	6.2
ZSH 22 (Rwerere)	10.9	5.6	6.8	4.3
Achyut	11.3	14.8	9.9	5.8
Ocepar 7	15.6	8.7	12.8	8.8
BH 1146	12.7	8.9	12.6	2.1
BL 1813	19.8	5.2	4.5	25.6
NL 872	6.0	14.5	12.1	12.7
NL 835	10.7	15.2	13.7	8.8
Triveni	12.3	9.8	10.3	5.1
NL 588	20.4	16.5	12.6	5.0
Rohini	13.8	14.3	17.5	7.0
BL 1692	18.3	14.9	12.6	3.4
Fang 60	12.5	13.9	16.0	17.8
Bhrikuti	8.3	15.0	12.8	7.5
BL 1724	12.4	14.8	11.8	2.8
NL 788	4.7	13.9	24.0	5.4
NL 753	14.7	24.2	29.5	0.5

Annapurna-1	24.1	5.7	12.6	7.9
Vaskar	9.3	23.4	28.1	12.2
Nirit	12.7	19.9	20.4	5.5
Kavkaz/K4500	12.4	13.0	27.4	7.7
NL 731	10.0	25.0	23.6	7.2
Kanchan	10.8	7.0	7.5	15.1
BL 1473	12.2	4.6	4.2	3.9
Nepal 297	14.0	7.6	4.4	5.6
BL 1530	12.5	19.7	24.9	7.7
Shafir	7.5	7.5	5.6	7.9
UP 262	13.2	7.8	5.7	4.3
Vinayak	13.5	10.7	28.7	16.2
Nepal 251	18.7	14.1	21.8	4.1
Siddhartha	13.9	21.0	26.5	12.2
Lumbini	20.6	17.8	16.5	13.1
BL 1022	9.7	7.2	6.8	9.7
HD1982	20.5	22.9	24.2	11.4
BL 1135	22.3	23.6	33.0	12.2
RR 21 (Sonalika)	18.6	25.6	27.6	16.2
Mean	11.2	11.0	11.7	7.7

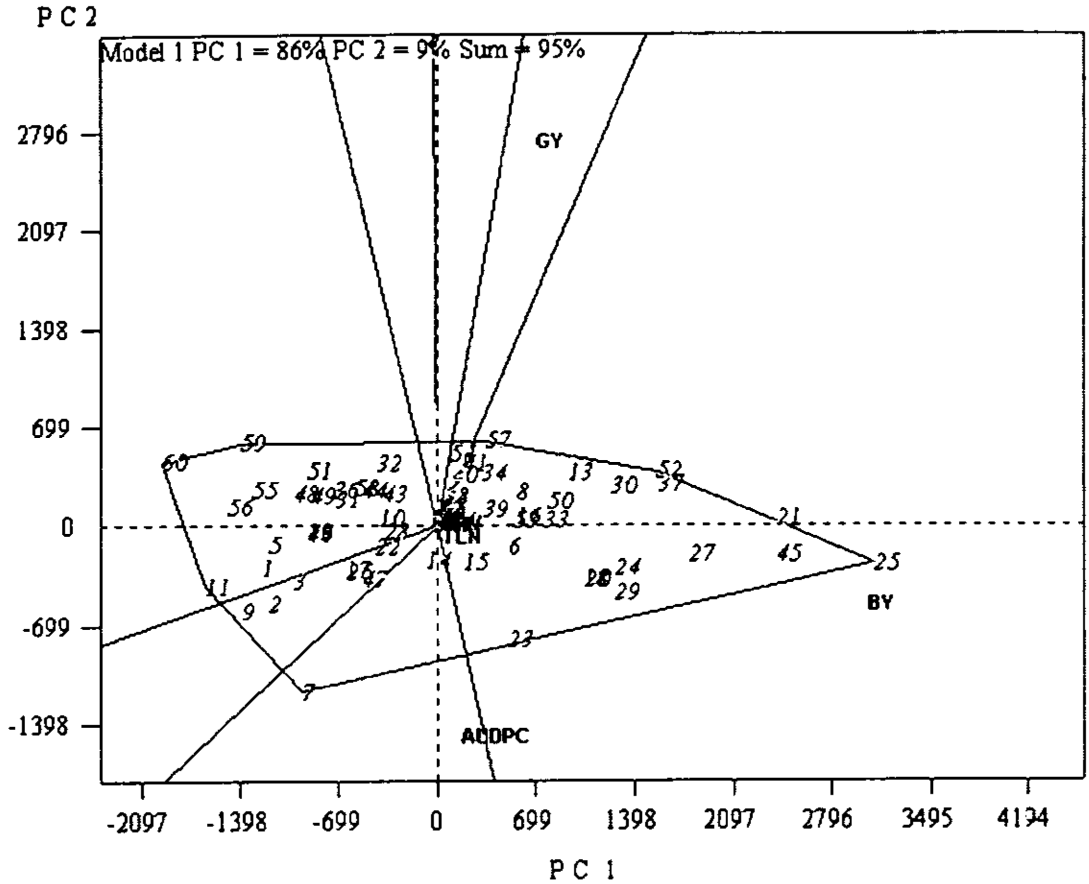

Figure 1. GGE biplot based on area under disease progress curve (AUDPC), biomass yield (BY), grain yield (GY), 1000-kernel weight (TKW), days to heading (DH), days to maturity (DM), plant height (PHT), and effective tiller number (TLN) of 60 wheat genotypes (refer to Table 1) evaluated at two sites in Nepal in 1999 and 2000.

Appendix 2. Genetic control of resistance to Helminthosporium leaf blight in wheat

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Wheat breeders in South Asia are continuously attempting to develop wheat (*Triticum aestivum* L.) cultivars resistant to Helminthosporium leaf blight (HLB). Information on the genetic control and suitability as breeding parents for HLB resistance in wheat cultivars of South Asia is not available. A study was undertaken to examine the resistance to HLB of nine genetically diverse wheat parents, and to evaluate their general combining ability (GCA) and specific combining ability (SCA) effects toward determining the genetic basis of disease resistance.

Methodology

Nine parents were crossed in a half-diallel mating design to produce 36 populations. The F_1 and F_2 progenies, and the parents were evaluated in replicated field tests at Rampur, Nepal. Multiple disease scores were recorded and area under the disease progress curve (AUDPC) was calculated to measure disease severity over time. The combining ability analysis was performed using Griffing's Method 2, Model 1.

Results

Parents showed wide variation for resistance to HLB (Table 3). The parents as well as F_1 and F_2 progenies significantly differed for AUDPC. GCA and SCA effects were significant in both generations suggesting that additive as well as non-additive gene actions were involved in the control of disease resistance in these parents. Wheat genotypes 'SW 89-5422', 'G 162' 'NL 781'and 'Chirya 7' had significantly negative GCA effect for AUDPC in both F_1 and F_2 (Table 3) generations suggesting their suitability in wheat breeding programs to improve resistance to HLB. Specific combining ability effects were generally non-significant (Table 4). Only 10 and 8 crosses out of 36 had significant SCA effects in F_1 and F_2 generation, respectively. The estimate of narrow sense heritability was 0.77 in both generations suggesting that selection for HLB resistance could be effective in these crosses (Table 5). The results suggest predominance of additive gene action in the inheritance of HLB resistance.

Conclusion

In general, the nine wheat parents differed in disease resistance and genotypes exotic to the region had a higher level of resistance. The predominance of GCA effect for AUDPC indicates that resistance to HLB can be improved through selection. Parents with a high level of resistance and significant negative GCA estimates were identified but a certain level of inconsistency in GCA estimates was found in both generations. Based on AUDPC values and GCA estimates, SW89-5422, NL 781, G162 and Chirya 7 are likely superior sources for HLB resistance for further use in wheat breeding programs targeted to improving this trait. Among all parents, SW89-5422 was the best general combiner for resistance to HLB. The HLB-resistant parents involved in this study are now widely used in wheat breeding programs in South Asia as potential new sources of resistance against this important disease. Hence, the information on their suitability as breeding parents is valuable to wheat breeders toward developing crossing plans and selection schemes in view to improve the level of resistance to HLB in susceptible commercial cultivars.

Table 3. Parental mean and general combining ability (GCA) estimates for area under disease progress curve (AUDPC) of Helminthosporium leaf blight in the F_1 and F_2 generations from a nine parent diallel analysis in spring wheat.

Parent	AUDPC	GCA F_1	GCA F_2
Chirya 7	314 ef[†]	-38.0 **	-81.5 **
SW89-5422	49 h	-175.6 **	-239.9 **
G 162	280 fg	-95.1 **	-91.6 **
BL 1724	469 d	11.0	4.1
NL 781	210 g	-76.3 **	-117.8 **
Bhrikuti	604 c	45.5 **	42.8 *
Attila	374 de	-50.9 *	-1.4
HD 2329	758 b	146.4 **	206.9 **
Sonalika	947 a	233.2 **	278.5 **
Mean	445		
Correlation coefficient (r) between			
Mean and GCA		0.99 **	0.99 **
F_1 GCA and F_2 GCA		0.98**	

[†] Means followed by the same letter within a column are nonsignificantly different based on $LSD_{0.05}$

*, ** Significantly different from zero at P = 0.05 and 0.01, respectively.

Table 4. Cross mean and estimates of specific combining ability (SCA) for area under disease progress curve of Helminthosporium leaf blight in a nine-parent diallel analysis in spring wheat.

Cross		F_1		F_2	
Number	Name	Mean	SCA	Mean	SCA
1	Chirya 7 / SW 89-5422	65	-2.1	261	-7.7
2	Chirya 7 / G 162	128	-19.8	271	-147.5 **
3	Chirya 7 / BL 1724	262	8.2	469	-44.4
4	Chirya 7 / NL 781	133	-34.0	414	22.9
5	Chirya 7 / Bhrikuti	258	-30.9	503	49.3
6	Chirya 7 / Attila	177	-14.7	426	-81.2
7	Chirya 7 / HD 2329	457	67.8 *	764	47.9
8	Chirya 7 / Sonalika	585	120.2 **	955	167.6 **
9	SW 89-5422 / G 162	35	24.7	265	5.9
10	SW 89-5422 / BL 1724	69	-47.8	266	-87.3
11	SW 89-5422 / NL 781	76	47.2	246	13.4
12	SW 89-5422 / Bhrikuti	138	27.3	370	-23.6
13	SW 89-5422 / Attila	82	27.8	431	81.5
14	SW 89-5422 / HD 2329	172	-80.2 *	682	124.3 **
15	SW 89-5422 / Sonalika	187	-140.1 **	586	-43.3
16	G 162 / BL 1724	280	82.7 **	591	87.3
17	G 162 / NL 781	97	-12.6	274	-107.3 *
18	G 162 / Bhrikuti	159	17.2	466	-76.5
19	G 162 / Attila	112	-22.9	545	28.6
20	G 162 / HD 2329	298	-34.3	736	29.6
21	G 162 / Sonalika	366	-42.1	818	40.3
22	BL 1724 / NL 781	309	92.8 **	594	116.8 *
23	BL 1724 / Bhrikuti	329	-8.6	640	2.2
24	BL 1724 / Attila	244	3.1	530	63.1
25	BL 1724 / HD 2329	292	-146.5 **	708	-93.9
26	BL 1724 / Sonalika	534	20.0	875	1.6
27	NL 781 / Bhrikuti	306	23.0	600	84.4
28	NL 781 / Attila	138	-15.7	408	-81.3
29	NL 781 / HD 23329	374	22.7	777	6.1
30	NL 781 / Sonalika	312	-114.6 **	697	-54.3
31	Bhrikuti / Attila	261	14.7	628	3.7
32	Bhrikuti / HD 2329	481	7.6	681	-159.2 **
33	Bhrikuti / Sonalika	489	59.4	887	-24.6
34	Attila / HD 2329	299	-77.9 *	967	168.6 **
35	Attila / Sonalika	454	2.1	923	137.1 **
36	HD 2329 / Sonalika	822	172.1 **	1048	-28.1
	Mean	272		592	
	$LSD_{0.05}$	52		94	

*,** Significantly different from zero at P = 0.05 and 0.01, respectively.

Table 5. Estimates of additive (σ^2_A), non-additive (σ^2_{NA}), and environmental (σ^2_E) components of variance, and narrow sense (h^2_n) and broad-sense (h^2_b) heritability for area under disease progress curve of Helminthosporium leaf blight in a nine-parent diallel analysis in spring wheat.

Genetic parameter	F_1	F_2
σ^2_A	15,550	25,398
σ^2_{NA}	3,671	4,360
σ^2_E	1,038	3,360
h^2_n	0.77	0.77
h^2_b	0.95	0.90

Table 6. Inheritance of early flowering in wheat crosses grown at Rampur, Nepal in 1998-99 wheat growing season.

Cross	Maturity Early	Late	Ratio tested (Early:Late)	χ^2 Probability
Vinayak x HLB 25	152	43	3 : 1	0.342
Chirya 7 x Ciano 79	177	77	3 : 1	0.050
SW-89-3060 x HD 2329	218	69	3 : 1	0.708

Table 7. Inheritance of resistance to HLB in wheat crosses under natural infection at Rampur, Nepal in 1998-99 wheat growing season.

Cross	Resistant	Susceptible	Ratio tested (Res. : Sus.)	χ^2 Probability
Vinayak x HLB 25	144	51	3 : 1	0.710
Chirya 7 x Ciano 79	196	58	3 : 1	0.426
SW-89-3060 x HD 2329	215	72	3 : 1	0.973

Table 8. Inheritance of early flowering and resistance to HLB in the F_2 generation of wheat crosses under natural infection at Rampur, Nepal in 1998-99 wheat growing season.

	Cross		
	Vinayak x HLB 25	Chirya 7 x Ciano 79	SW-89-3060 x HD 2329
Early - Resistant (ER)	114	138	164
Early - Susceptible (ES)	38	39	54
Late - Resistant (LR)	30	58	51
Late - Susceptible (LS)	13	19	18
Ratio tested	(9 ER : 3 ES : 3 LR : 1 LS)		
χ^2 - probability	0.691	0.203	0.979

Appendix 4. Selection index for improving Helminthosporium leaf blight resistance, maturity and kernel weight in spring wheat

[Published in Crop Science 43:2031-2036 (2003)]

A selection strategy is needed to identify early maturing, HLB-resistant genotypes, given that most early maturing wheat cultivars in South Asia are either susceptible or have low levels of resistance to Helminthosporium leaf blight (HLB). A study was conducted to determine whether three traits (resistance to HLB, maturity, and kernel weight) could be simultaneously improved with a selection index (S.I.) combining the area under disease progress curve (AUDPC) as an assessment of disease severity, days to heading (DHD), and thousand-kernel weight (TKW).

Methodology

An early maturing and HLB-susceptible cultivar 'Sonalika' was crossed to four late maturing HLB-resistant wheat genotypes to develop four populations used in this study. The four F_2-derived F_3 families and both parents of each cross were grown in 1-m long single row plots. In each row, 100 seedlings were maintained to obtain a solid crop stand. Multiple HLB scores were taken (double digit 00-99 scale) to calculate the area under disease progress curve (AUDPC) per progeny. Days to heading (DH) and thousand-kernel weight (TKW) were also recorded. The following selection index (S.I.) using AUDPC, DH, and TKW was developed:

S.I. = (AUDPC rank in ascending order + DH rank in ascending order) + TKW rank in descending order)

In practice, the F_3 line with the lowest AUDPC value was given a rank value of 1. Similarly, the F_3 line with the lowest DH value was ranked 1. In contrast, the F_3 line with the highest value for TKW was ranked 1.

In each of the four crosses, 20 lines with the lowest index and the 20 with the highest index were selected among a total of 234 F_3 progenies. The 40 lines selected in each cross were evaluated during the 2001-2002 growing season, in a replicated field test conducted under natural inoculum pressure at two sites of the lowlands of Nepal (Manara and Rampur) where HLB severity has been high on wheat crop for the past several years. Both trials were planted and managed following optimum crop husbandry practices recommended for the area.

Three HLB scores were recorded during the post-anthesis period using the double-digit system and the AUDPC was calculated after conversion to estimate the percent diseased leaf area. The other data recorded were DH, TKW, biomass yield (BY), grain yield (GY), harvest index (HI), and plant height (PHT). To determine the effectiveness of the selection index the significance of difference between high and low selection groups for the traits included in the selection index was tested. Correlated responses were measured in terms of changes brought in other important characters, such as GY, BMY, HI, and PHT.

To be of practical significance to wheat breeders, it is important that desirable response be observed for GY and PHT. The best lines identified should have high GY, low AUDPC, high TKW, early heading and acceptable PHT.

Results

Selection in the F_3 generation using the low and high S.I. was effective in identifying F_4 lines with low and high AUDPC, respectively. The use of low S.I. was associated with higher grain yield and higher TKW, without significantly affecting DHD and plant height. The AUDPC was reduced by 579 to 837, depending on location and population, while TKW was increased by 7.8 to 12.7 g, and grain yield by 786 to 1491 kg ha^{-1} (Table 9). The use of S.I. also produced positive response in biomass and grain yields. There was an average 43% increase in grain yield of the low S.I. group compared with the high S.I. group. The F_3 lines belonging to the low and high S.I. categories remained in their respective S.I. groups in the F_4 generation, indicating that environmental conditions did not have a substantial effect on S.I. The results suggested that selection for early maturing, HLB-resistant wheat lines with high grain yield and kernel weight is possible using a S.I.

Conclusion

This is the first report on the use of a simple S.I. to improve HLB resistance and maturity in wheat. It is likely to prove particularly useful to wheat breeders who usually base genotype selection on means and ranks. Since the three characters that are part of the S.I. in this study are recorded regularly in most wheat breeding programs, the use of an S.I. does not increase workload. One could argue that using truncated selection could be as good as or even simpler than using an S.I.; however, breeders using truncated selection would be tempted to select first for early heading, then for disease resistance and, finally, for kernel weight. Truncated selection would thus consider maturity more important than disease resistance and TKW. A breeder might consider selecting a line showing high levels of HLB resistance and high TKW with even intermediate maturity. This flexibility is inherent to the proposed S.I. and cannot be done using truncated selection. The advantage of using an S.I. is that it is flexible enough to allow balancing moderate defects in one trait with obvious gain in others.

Table 9. Area under disease progress curve (AUDPC), days to heading, 1000-kernel weight, biomass yield, grain yield, harvest index, and plant height of F_4 lines following an index selection for AUDPC, days to heading, and 1000-kernel weight in the F_3 generation in four wheat populations.

			Mean of low (L) and high (H) selection index groups													
			AUDPC		Days to heading		1000-kernel weight		Biomass yield		Grain yield		Harvest index		Plant height	
Pop†	Gen‡	Location	L	H	L	H	L	H	L	H	L	H	L	H	L	H
							------- g ------		---------------- kg ha^{-1} -------------				------- % -------		--- cm ---	
1	F3	Rampur	192	1016	74	71	54.0	36.2	-	-	-	-	-	-	-	-
	F4	Rampur	132**	969	74	73	49.1**	39.9	8481**	6720	3847**	2902	45.4	43.2	86	86
	F4	Manara	106**	820	72	70	53.3**	43.0	9306**	6924	4572**	3081	49.1*	44.5	89	92
2.	F3	Rampur	144	986	76	72	51.3	37.0	-	-	-	-	-	-	-	-
	F4	Rampur	77**	842	72	72	44.5**	34.1	8125**	6706	3409**	2555	42.0*	38.1	90	93
	F4	Manara	83**	805	73	71	46.3**	33.6	9662**	7656	3929**	2692	40.7**	35.2	95	96
3	F3	Rampur	207	924	77	73	53.5	39.2	-	-	-	-	-	-	-	-
	F4	Rampur	158**	801	72	71	47.9**	39.6	7309**	5836	2272**	1486	31.1**	25.5	77	81
	F4	Manara	134**	713	71	71	51.8**	41.8	7947**	5948	3230**	2081	40.6**	35.0	81	84
4.	F3	Rampur	214	1020	75	72	49.6	35.5	-	-	-	-	-	-	-	-
	F4	Rampur	150**	841	73	71	46.7**	38.9	8772**	6882	4100**	2994	46.7	43.5	85	87
	F4	Manara	133**	738	72	71	48.4**	38.8	9930**	7418	4487**	3071	45.2*	41.4	88	91

*, ** Difference between low and high selection index groups significant at $P = 0.05$ and 0.01, respectively, based on '*F*' test.
† Pop = Population, ‡ Gen = Generation

Appendix 5. Incidence of *Bipolaris sorokiniana* and *Pyrenophora tritici-repentis* on wheat in the lowlands of Nepal

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Helminthosporium leaf blight (HLB) is a serious disease in warmer wheat growing environments in South Asia. It is difficult to control this disease complex because both pathogens may occur independently at similar or different growth stages of a wheat crop. A decade ago, it was generally accepted that *B. sorokiniana* prevailed mostly in the warm lowlands of Nepal corresponding to the Gangetic plains (<200 masl) whereas *P. tritici-repentis* was found in the cooler climate of the hills (>800 masl) and that both pathogens could be equally identified in a transition zone characterized by mid-hills and river basins. However, in the early 1990s, *P. tritici-repentis* was occasionally isolated in the warm and humid lowland of Nepal, where *B. sorokiniana* dominated typically, suggesting that tan spot was probably overlooked. This study was conducted to investigate the presence of both pathogens across the southern wheat belt of lowland Nepal also known as *Tarai*.

Methodology

In Nepal, wheat is grown from November to April during the winter season. The survey was conducted during four wheat growing seasons: 1997-1998 (1998), 1998-1999 (1999), 1999-2000 (2000) and 2000-2001 (2001) following the east-west highway of Nepal. It covered a distance of more than 1000-km and included all the 20 lowland districts. It underlines the importance of the *Tarai* belt for food security in the country and of controlling foliar blights in that region which coincides with the max 40 km wide plain bordering the Himalayas that is already part of the Gangetic plains. The sampled districts where most Nepal wheat is grown (>300,000 hectares) cover 17% of the country.

Each year, diseased leaf samples were collected from the farmers' fields when the wheat crop was in mid to late grain filling stage during the 2nd and 3rd week of March. At each site, diseased leaves were collected walking in a zigzag manner covering several hectares of wheat. In most cases, infected leaf samples were collected approximately from the same sites/villages every year. A total of 267, 690, 614, and 475 samples were collected in 1998, 1999, 2000, and 2001, respectively.

The infected leaves were incubated in the laboratory at 24°C for 24 to 48 hours under alternate light in Petri plates containing moist filter paper. The leaf samples were examined under a stereomicroscope to determine the presence of one or both pathogens based on conidia formation.

Results

Both *B. sorokiniana* and *P. tritici-repentis* were found on wheat crop grown across Nepal lowlands in all four years (1998 to 2001) (Table 10). Overall, the incidence of *P. tritici-repentis* was above 60% and increased over years whereas that of *B. sorokiniana* found in more than 50% of the samples in 1998 and 1999 appeared decreasing in 2000 and 2001. The occurrence of both pathogens was found to be highly variable. On a total of 267, 690, 614, and 475 infected leaf samples collected from *1998* to *2001*, *B. sorokiniana* conidia *were respectively observed in 51, 69, 28,* 11% leaf samples (Fig. 2). Surprisingly, a significant increase in the *P. tritici-repentis* frequency was observed with 63, 68, 84, and 82% of the samples harboring tan spot in the same period.

Though environmental conditions could not be monitored in details in this survey, the climatic information obtained at key sites suggested that differences exist between regions that may affect the relative frequency of both pathogens. Only a detailed weekly observation at the field level may help to further understand the epidemiology of HLB in *Tarai* and hills conditions. Beside weather, other environmental factors most likely predispose to the disease. If these constraints are properly identified, crop management could be part of a disease control scheme including genetic resistance.

Conclusion

The results of this study confirm that incidence of both HLB causing pathogens could change unpredictably across the Eastern Gangetic Plains of South Asia. Hence, a continuous monitoring of these pathogens is necessary in order to manage them effectively. Wheat breeding programs in South Asia need to incorporate resistance genes for these two pathogens, possibly from distinct pools, in order to develop cultivars with durable resistance to HLB.

Table 10. Incidence of *Bipolaris sorokiniana* and *Pyrenophora tritici-repentis* in the lowland districts of Nepal in four years

District	Number of wheat leaf samples containing the pathogen							
	Bipolaris sorokiniana				*Pyrenophora tritici-repentis*			
	1998	1999	2000	2001	1998	1999	2000	2001
Jhapa	0	33	8	4	5	31	46	25
Morang	3	27	10	8	5	9	22	25
Sunsari	12	27	8	8	18	8	24	25
Saptari	6	30	11	3	6	3	25	24
Siraha	1	29	12	4	7	2	20	25
Dhanusha	8	30	17	2	9	23	16	24
Mahottari	9	3	6	0	9	22	6	23
Sarlahi	4	28	14	1	4	24	19	25
Rautahat	5	24	10	1	3	21	20	25
Bara	7	22	9	-	1	27	20	-
Parsa	5	-	6	-	2	-	21	-
Makwanpur	3	9	8	6	3	26	17	13
Chitwan	20	47	10	-	2	48	25	-
Nawalparasi	12	52	8	2	18	49	43	48
Rupandehi	32	0	26	-	41	30	32	-
Kapilbastu	1	16	0	0	5	30	22	13
Dang	4	21	5	4	13	30	22	16
Banke	3	4	2	2	11	24	24	20
Bardia	-	19	0	4	-	21	24	18
Kailali	1	39	5	5	6	21	46	40
Kanchanpur	-	13	1	-	-	19	24	-
Total	136	473	176	54	168	468	518	389
Total Samples	267	690	614	475	267	690	614	475
Frequency (%)	51	69	28	11	63	68	84	82

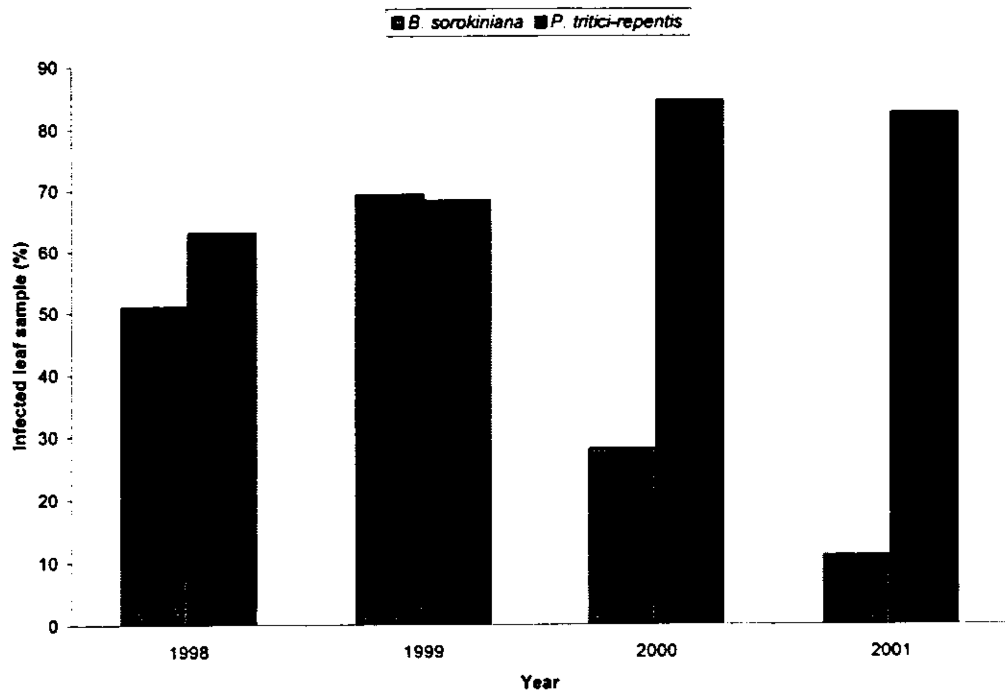

Figure 2. Frequency of HLB Pathogens in the lowlands of Nepal during 1998-2001 Surveys.

Appendix 6. Characterization of Helminthosporium leaf blight resistance in wheat at different growth stages

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Helminthosporium leaf blight (HLB), occurring either singly or as a complex of spot blotch, caused by *Bipolaris sorokiniana* (Ito & Kuribayashi) Drechs. ex Dastur, and tan spot, caused by *Pyrenophora tritici-repentis* (Died.) Drechs, is the most serious disease constraint to wheat (*Triticum aestivum* L.) yields in the warmer plain areas of South Asia. There is little documentation of epidemiological study in the rice-wheat system, elucidating in details when the disease really starts and how fast both pathogens multiply on wheat crop under different environmental conditions in terms of temperature and humidity. This study was conducted to: (i) examine initiation and tissue proliferation of *B. sorokiniana* and *P. tritici-repentis* throughout the wheat season on contrasting genotypes seeded on optimum and later than optimum dates; (ii) observe aerial incidence of the two pathogens during wheat cropping season, (iii) evaluate the possible inoculum source of both pathogens, and (iv) to determine the extent of grain yield loss by HLB.

Methodology

An experiment conducted in 2001-2002 (2002) and 2002-2003 (2003) at Rampur, Nepal (228-masl, 27°40' longitude, and 84°19' latitude). Six genetically diverse genotypes (RR21=Sonalika, BL1473, Nepal 297, NL750, NL781, Milan/Shanghai-7) differing in maturity and level of resistance to HLB were sown in four replicates, at three dates ranging from optimum (Nov. 26) to late sown (Dec 26) conditions. Sonalika, BL1473, and Nepal 297 are more susceptible to HLB compared to the other three varieties harboring high levels of resistance. The field trial followed a strip-split plot design with seeding date and fungicide spray as two strips and the six varieties as split factor. The individual plot size was 2-m x 1-m using a seed rate of 120 kg/ha and 0.25-m row spacing. The plots were managed following optimal crop husbandry recommendations for the region. In each plot, twenty plants were sampled at weekly intervals, starting one week after emergence. After incubation in the laboratory, the occurrence of spot blotch and tan spot was determined based on symptom observation under the stereo-binocular Fungal isolations were conducted to confirm the observations. Moreover, the amount of air-borne conidia of both pathogens was monitored on a weekly basis. Conidia were trapped during 17 weeks using a Rotorod® Model 20 sampler installed in the research plot.

After anthesis, four HLB scores were visually recorded using the double-digit scale (00-99) for each plot. When using the double-digit scoring system, disease severity percentage was estimated from the product of both digits, each one previously divided by 9 and multiplied by 100 [i.e. $(D_1/9) \times (D_2/9) \times 100$]. The area under the disease progress curve (AUDPC) was calculated using the percent severity estimates corresponding to the four ratings as shown below:

$$AUDPC = \sum_{i=1}^{n}\left[\left(Y_i + Y_{i+1}\right)/2\right]\left(X_{i+1} - X_i\right),$$

where, Y_i = HLB severity on the *i*th date, X_i = *i*th day, and n = number of dates on which HLB was recorded. The AUDPC was standardized by dividing by the total number of days of the assessment period (AUDPC/day) in order to directly compare among epidemics of different lengths for three seeding dates. The AUDPC was also standardized by dividing by the total number of degree days (AUDPC/DD) for making comparisons among epidemics related to different temperatures effects for the three seeding dates. . In Nepal conditions, average temperature during the disease assessment period are lower for timely seeded compared to late seeded wheat. Hence, the amount of degree days for an assessment period was calculated as the sum of daily mean temperature (°C) during the corresponding period. Grain yield (GY), thousand-kernel weight (TKW) and other agronomic data were recorded on a per plot basis.

The statistical analysis included an analysis of variance (ANOVA) to test main and interaction effects. The number of leaf samples showing the presence of *B. sorokiniana* and *P. tritici-repentis* was plotted for each variety against successive weeks of observation, to evaluate the early incidence and multiplication of the both pathogens on leaf tissues and determine if fungi development was affected by genotypes. Similarly, the number of conidia of both pathogens counted on the Rotorod® sampler was plotted to determine when they can be detected in the air and at what density. The graphs were compared to infer on possible sources of inoculum of two pathogens.

Results

B. sorokiniana and *P. tritici-repentis* were isolated from leaf samples taken at seedling and tillering stage on Dec. 8 and Feb. 2, respectively. Tissue infection was slow for four to six weeks and rapidly increased with increased temperature (Fig 3). The first air-borne conidia of *B. sorokiniana* and *P. tritici-repentis* were recorded in the week of January 20-27, and the conidia counts were much higher for *B. sorokiniana* than for *P. tritici-repentis*. Both pathogens had the maximum number of conidia in the air during the last week of March and the 1st week of April. Results suggest that seed could be the primary source of inoculum for *B. sorokiniana* and that air-borne conidia are related to the profuse multiplication during secondary infection cycles, whereas the infection by *P. tritici-repentis* might result primarily from the air-borne spores originating probably on alternate hosts. HLB caused an average 30% reduction in grain yield with higher losses under delayed seeding. Increase in AUDPC per day or per degree-day better explained the potential yield losses compared to AUDPC per se. The results of this study are first comprehensive report on epidemiology of foliar blight pathogens in South Asia, especially for rice-wheat system. The findings underline the important effect of various climatic factors on HLB development and could lead to new guidelines for improving manipulation of climatic information as part of an integrated crop management strategy.

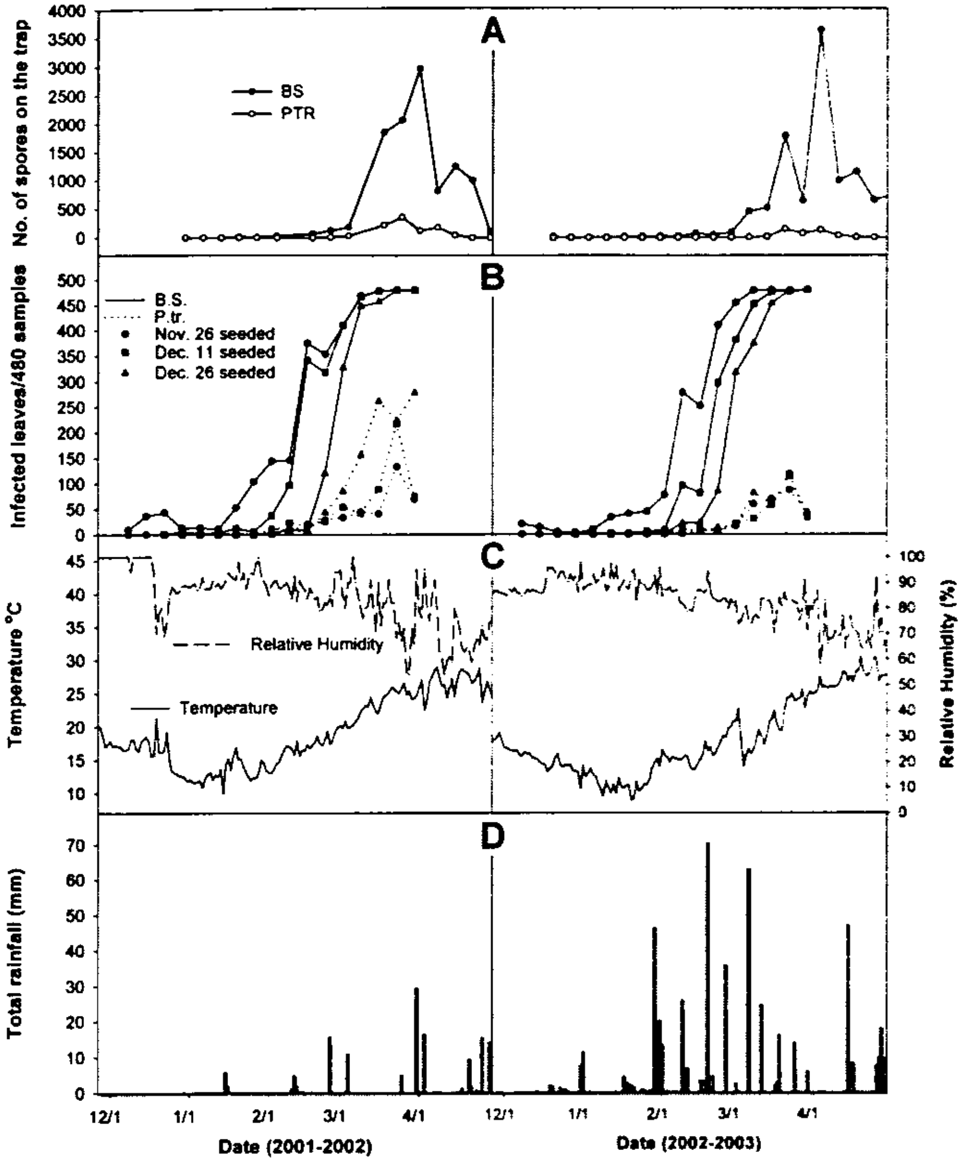

Figure 3. Number of conidia in air (A), frequency of leaf number infected with pathogens (B), mean daily air temperature and humidity (C) and daily rainfall recorded during the wheat crop cycle at Rampur Nepal in two years.

Table 11. Mean values for area under disease progress curve (AUDPC), AUDPC per day (AUDPC/day) and per degree day (AUDPC/DD) under three seeding dates in 2002 and 2003 wheat growing seasons averaged over six genotypes.

Seeding date	AUDPC		AUDPC/day		AUDPC/DD	
	2002	2003	2002	2003	2002	2003
26 November	467 ab†, A‡	356 c, B	25 c, B	19 c, A	1.2 c, A	0.9 c, B
11 December	515 a, A	397 bc, B	40 b, A	31 b, B	1.7 b, A	1.5 b, A
26 December	424 c, B	484 a, A	47 a, B	54 a, A	1.9 a, B	2.4 a, A
Mean	469 A	412 B	37 A	35 B	1.6 A	1.5 A

† Means within a column followed by the same lower case letter do not differ significantly based on $LSD_{0.05}$.
‡ Means for a given trait in a row for the same planting date followed by the same upper case letter do not differ significantly in the two years based on $LSD_{0.05}$

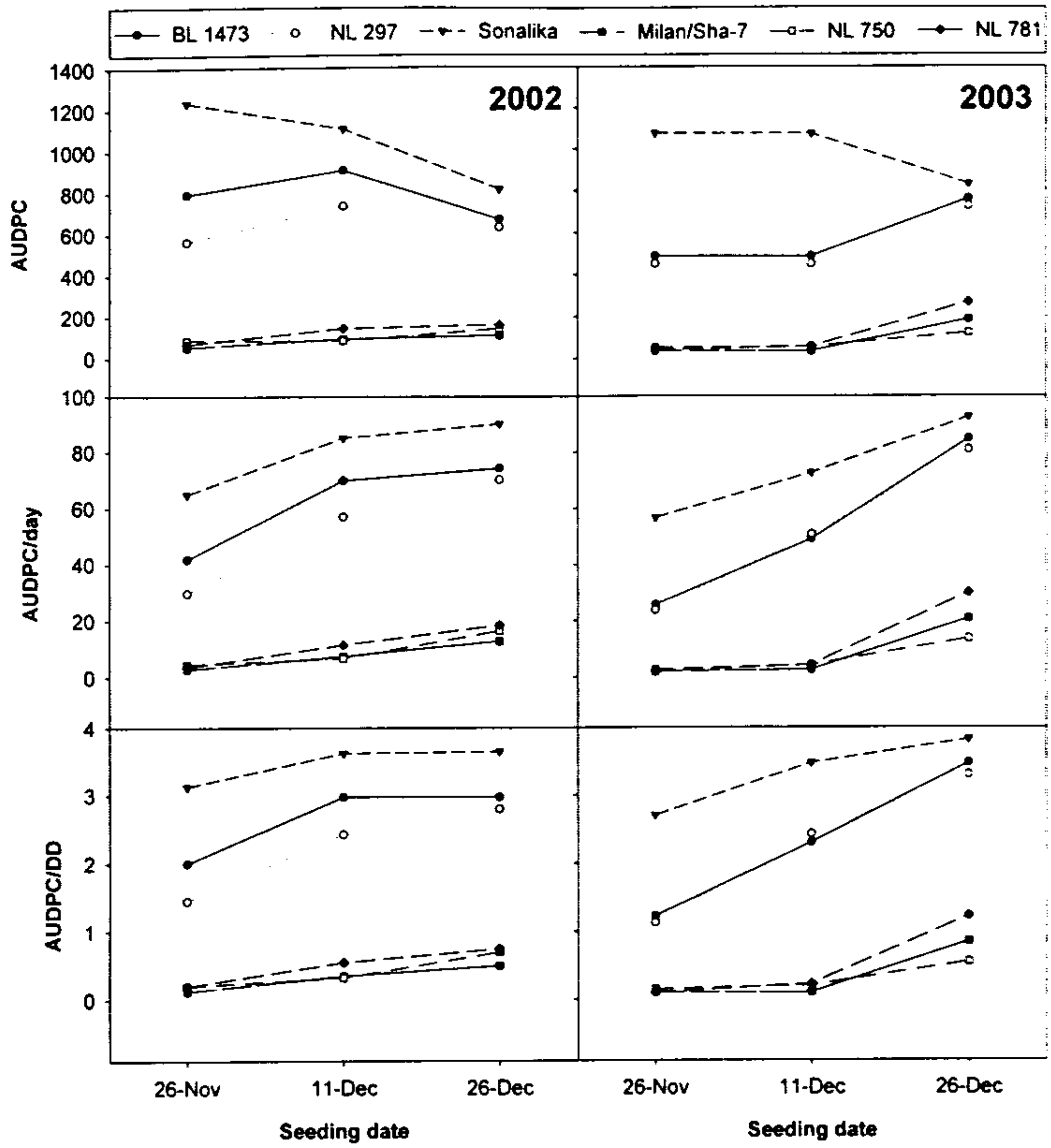

Figure 4. Change in AUDPC, AUDPC per day (AUDPC/ay) and AUDPC per degree day (AUDPC/DD) in six wheat genotypes over three seeding dates in 2002 and 2003 wheat season at Rampur, Nepal.

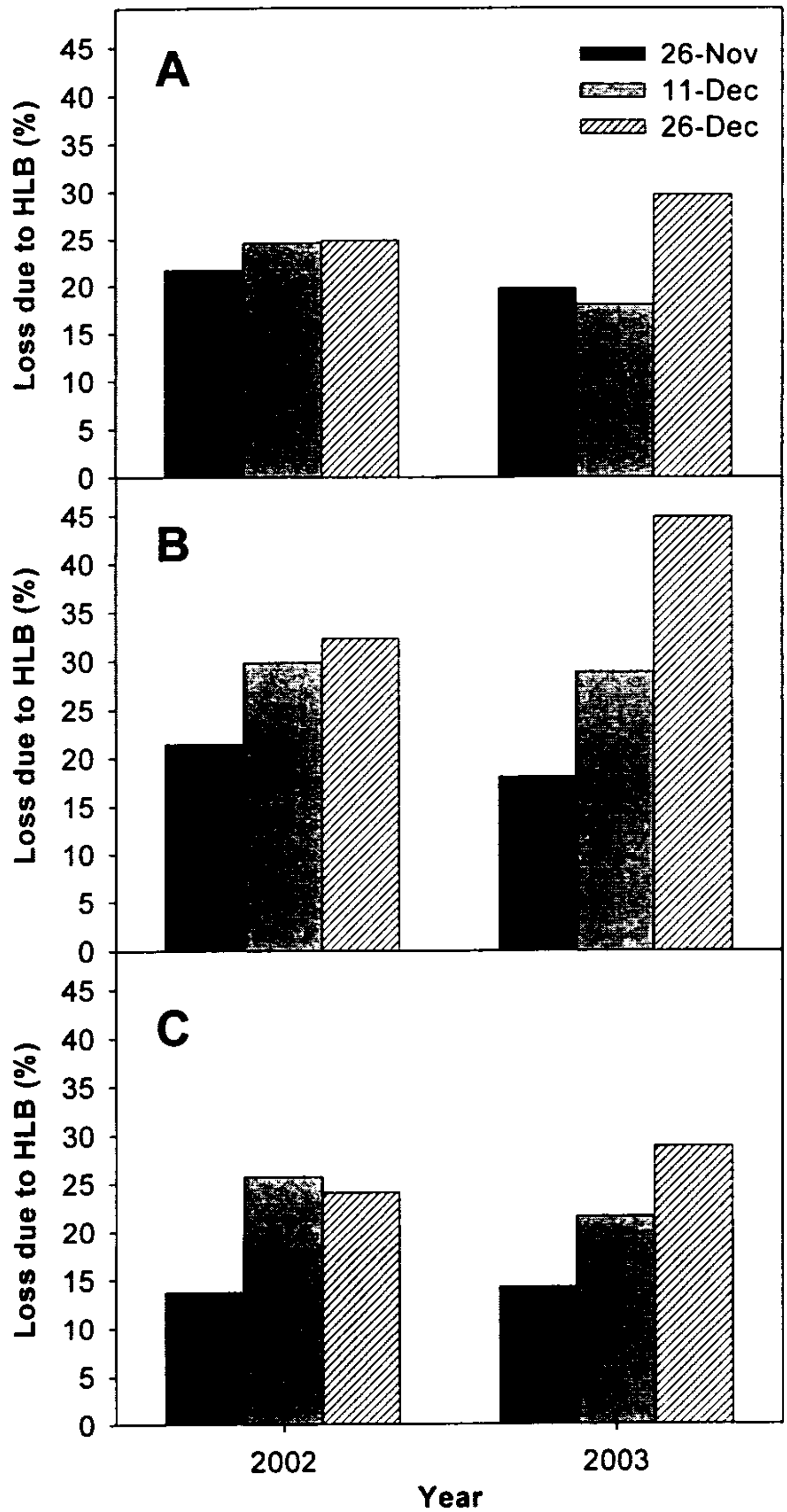

Figure 5. Reduction in biomass yield (A), grain yield (B), and thousand-kernel weight (C) due to Helminthosporium leaf blight under three seeding dates of wheat in two years.

Appendix 7. Combining tolerance from locally adapted and resistance from exotic wheat genotypes and evaluating performance of progeny lines

This study was conducted to identify progenies with higher levels of resistance to Helminthosporium leaf blight (HLB) resulting from crossed involving tolerant local cultivars and exotic resistant genotypes. Besides resistance agronomic characters were also considered in identify superior progeny. T

Methodology

Twenty F_5 lines from the crosses involving highly resistant parents were identified based on the past year's study on both farmer's field and research station. These lines along with commercial wheat varieties as check were tested in replicated field tests at a farmer's field and research station to determine level of genetic tolerance and resistance of these lines. This will be fourth season of these lines being exposed to natural population of the fungi. Besides disease resistance and high yield, these 20 lines were also subject to farmers' selection criteria. The results are presented below. Mean agronomic performance at research station Rampur, and a farmer's field at Janakpur are given in the Table 12 below.

Results

All the selections showed lower disease severity (Table 12). For most of selections, the level of disease was lower than the resistant parent involved in the cross. It shows that transgressive segregates were selected for disease resistance. This also indicates that in many of the resistant progenies, there were combinations of adaptive genes from the local susceptible parents with background tolerance to disease and frontal resistance from the unadapted parents. Some of these progenies could possess durable resistance to foliar blight.

Grain yield of the selected progenies ranged from low to high compared to the highest yielding check cultivar. However, the progenies always out-yielded the parents. Several selections (Sel 1, 5, 7, 12, 14, 15 and 18) were high grain yielding compared to the highest yielding checks (BL1887 and BL1473).

All selections had early maturity, an important trait for farmers' acceptability of a wheat variety in the warm regions of South Asia. Many selections had thousand-grain weight comparable to the best check cultivars. Most of the selections also had acceptable plant height.

The farmers' preference rank of many selections was low primarily because of segregation of one or more traits. Segregation for certain visible morphological characters such as chaff color and plant height occurred within a plot of selected progeny because all of the selected progenies were F_2-derived lines. Such progenies could be purified by bulk selection before further testing. However, a few selections (Sel 1, 2, 3, 14, 16) were preferred by farmers over a number of commercial varieties.

The findings of this study suggest that high grain yielding disease resistant progenies were identified which could be further tested in broader yield trials.

Table 12. Selection/ Genotype	Pedigree	AUDPC Rampur	AUDPC Janakpur	Days to heading Rampur	Days to heading Janakpur	Grain yield Rampur	Grain yield Janakpur	Thousand kernel weight Rampur	Thousand kernel weight Janakpur	Plant height Rampur	Plant height Janakpur	Farmers' Rank Rampur
						-------- kg/ha -----		------- g --------		------- cm -------		
Sel 1	(Sonalika/Chirya 7)	185	112	70	74	[redacted]	[redacted]	47.2	52.5	97	106	7
Sel 2	(Sonalika/Chirya 7)	174	122	69	72	4120	4360	49.9	52.4	100	111	3
Sel 3	(Sonalika/Chirya 7)	218	154	71	73	3472	4704	48.7	52.1	98	107	6
Sel 4	(Sonalika/Chirya 7)	219	152	69	71	3806	3900	47.1	48.2	99	108	22
Sel 5	(Sonalika/Chirya 7)	194	143	71	73	[redacted]	[redacted]	48.6	50.0	94	104	9
Sel 6	(Sonalika/SW89-5422)	163	159	72	74	3407	3938	43.3	43.7	98	110	25
Sel 7	(Sonalika/SW89-5422)	121	88	71	74	3758	[redacted]	46.7	49.8	89	100	12
Sel 8	(Sonalika/SW89-5422)	138	123	69	74	3506	3796	49.0	50.8	95	106	15
Sel 9	(Sonalika/SW89-5422)	135	115	70	73	3950	4302	46.6	48.5	94	101	13
Sel 10	(Sonalika/SW89-5422)	171	140	69	72	3684	4099	44.2	46.3	97	105	24
Sel 11	(Sonalika/G 162)	176	158	72	74	3568	3785	43.3	44.0	84	89	26
Sel 12	(Sonalika/G 162)	170	135	69	71	[redacted]	[redacted]	47.8	52.5	84	96	11
Sel 13	(Sonalika/G 162)	188	117	70	74	3489	4448	46.6	46.3	84	91	21
Sel 14	(Sonalika/G 162)	182	135	69	72	[redacted]	[redacted]	48.5	52.9	87	94	4
Sel 15	(Sonalika/G 162)	179	120	69	72	[redacted]	[redacted]	49.4	52.0	85	91	8
Sel 16	(Sonalika/Attila)	206	139	72	75	3867	4314	45.4	47.5	89	92	5
Sel 17	(Sonalika/Attila)	191	151	68	72	3793	4446	45.1	48.6	83	92	20
Sel 18	(Sonalika/Attila)	215	162	70	74	3752	[redacted]	47.1	51.2	85	90	9
Sel 19	(Sonalika/Attila)	194	140	71	75	3935	4554	47.5	49.0	85	92	14
Sel 20	(Sonalika/Attila)	213	151	71	73	3641	4494	44.8	47.4	86	93	19
Attila	(Check)	238	188	86	88	2973	3589	34.3	34.8	85	93	28
Chirya 7	(Check)	237	146	83	87	3333	3679	35.3	35.5	103	108	23
G162	(Check)	245	204	84	89	2015	2523	35.7	36.1	84	95	30
SW89-5422	(Check)	163	134	83	86	3129	3238	33.9	37.4	97	100	29
BL1473	(Check)	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	2
BL1887	(Check)	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	1
Bhrikuti	(Check)	512	434	76	79	3691	4450	43.5	45.1	89	100	16
Kanchan	(Check)	841	782	71	73	2877	3866	44.6	45.6	102	108	18
Nepal 297	(Check)	906	790	71	71	2416	3268	49.4	51.2	100	107	17
Sonalika	(Check)	1166	1047	70	73	1872	2078	41.1	43.6	102	109	27

Appendix 8. Variability in *Bipolaris sorokiniana* and host resistance

This study was conducted to determine variation in *Bipolaris sorokiniana* isolates collected from different parts of Nepal. Genetic variation among pathogen is key to determine the effectiveness of host resistance gene and their geographical deployment.

Methodology

Two studies were conducted to determine the variation in pathogen based on their reaction to 10 wheat genotypes of diverse genetic background possessing different levels of foliar blight resistance.

The first study was conducted in the greenhouse where 10 wheat genotypes were inoculated with 20 single spore isolates of *B. sorokiniana*. The study was set in a Randomized Complete Block with three replicates. Twenty-one day old seedlings were sprayed with spore suspension of individual isolates and scored for infection one week after inoculation.

The second study involved inoculation of detached leaf if 10 wheat genotypes with spore suspension of 41 isolates of *B. sorokiniana*. The inoculated leaf pieces were incubated in the lab for one week before recording the symptoms.

Results

The 10 wheat genotypes showed diverse reaction to the isolates of *B. sorokiniana* (Fig. 6 and 7). The grouping of the 10 genotypes into different clusters suggested diverse resistance genes among the host genotypes.

There was a wide variation among isolates of *B. sorokiniana* evident through several clusters in which the isolates grouped in both studies (Fig. 8 and 9)

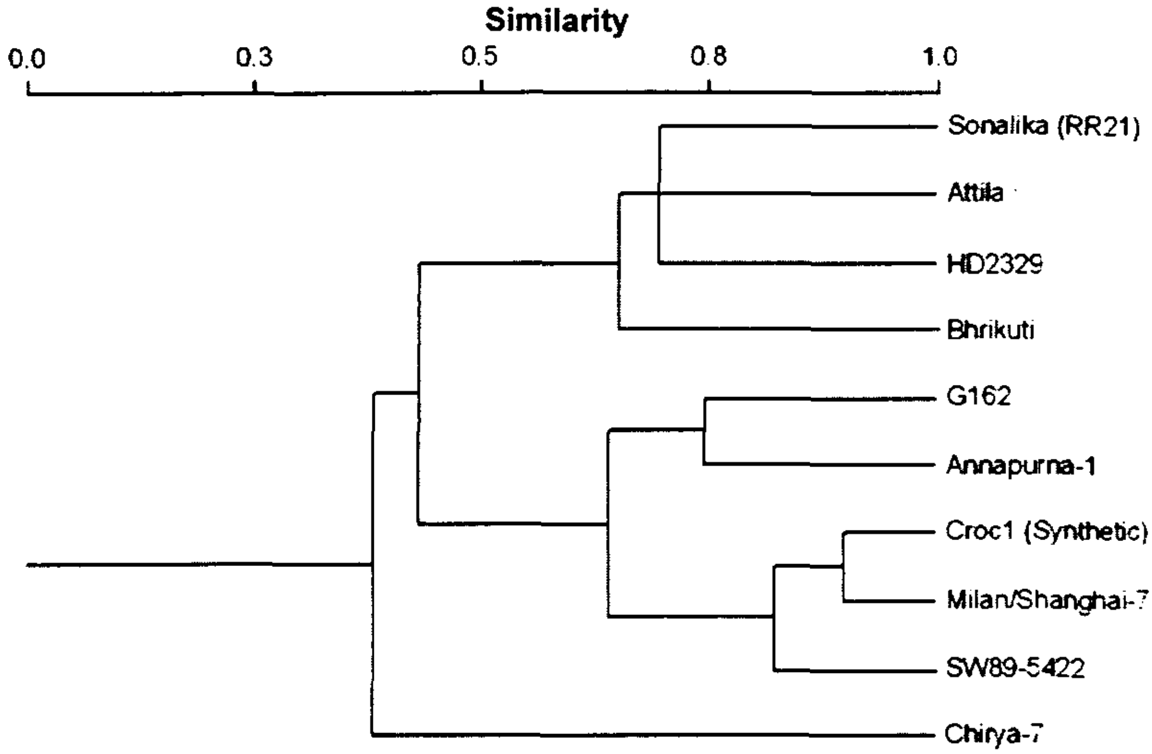

Figure 6. Clustering of 10 wheat genotypes based on their resistance to susceptible reaction to 20 isolates of *B. sorokiniana* in a greenhouse seedling inoculation test.

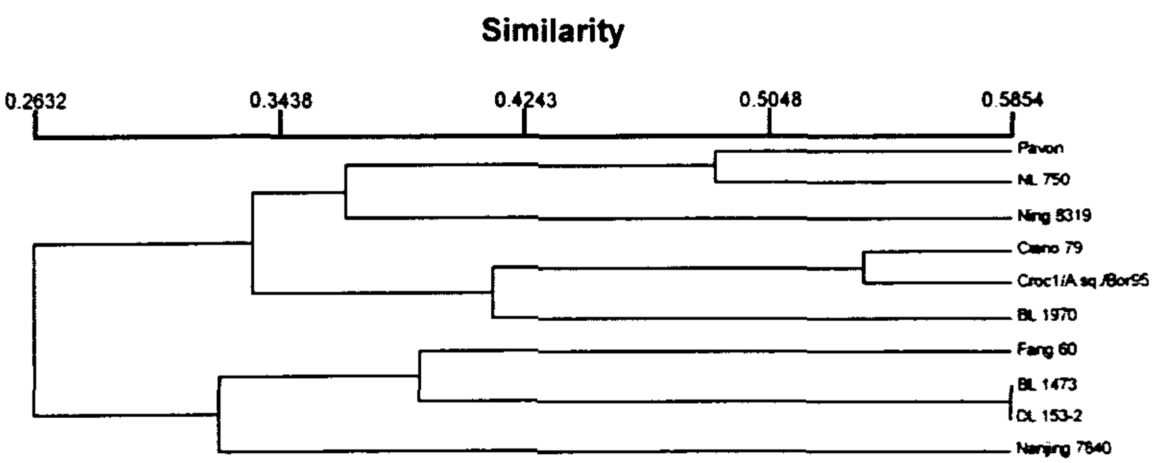

Figure 7. Clustering of 10 wheat genotypes based on their resistance to susceptible reaction to 41 isolates of *B. sorokiniana* in a detached leaf inoculation test.

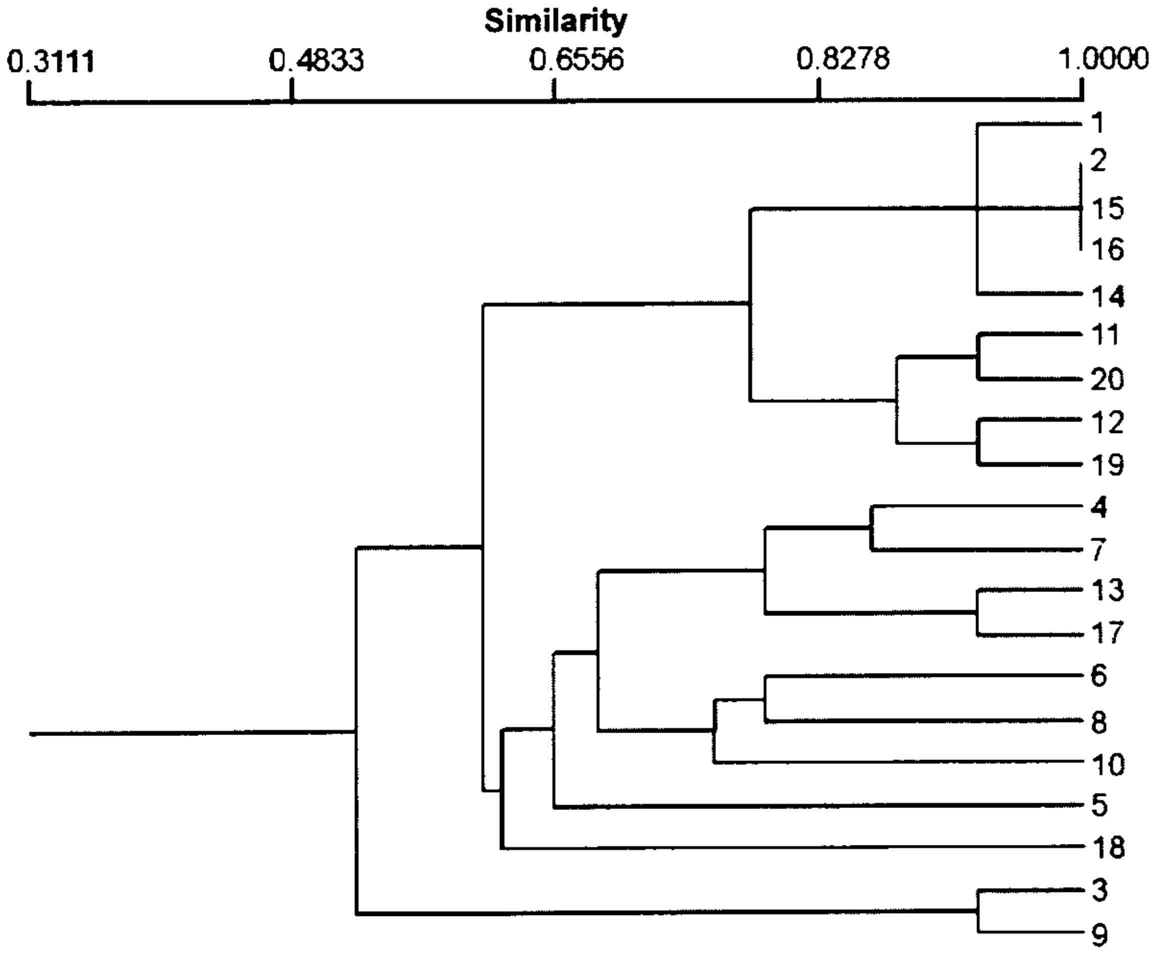

Figure 8. Clustering of 20 isolates of *B. sorokiniana* in a greenhouse seedling inoculation test on 10 wheat genotypes given in Fig. 6.

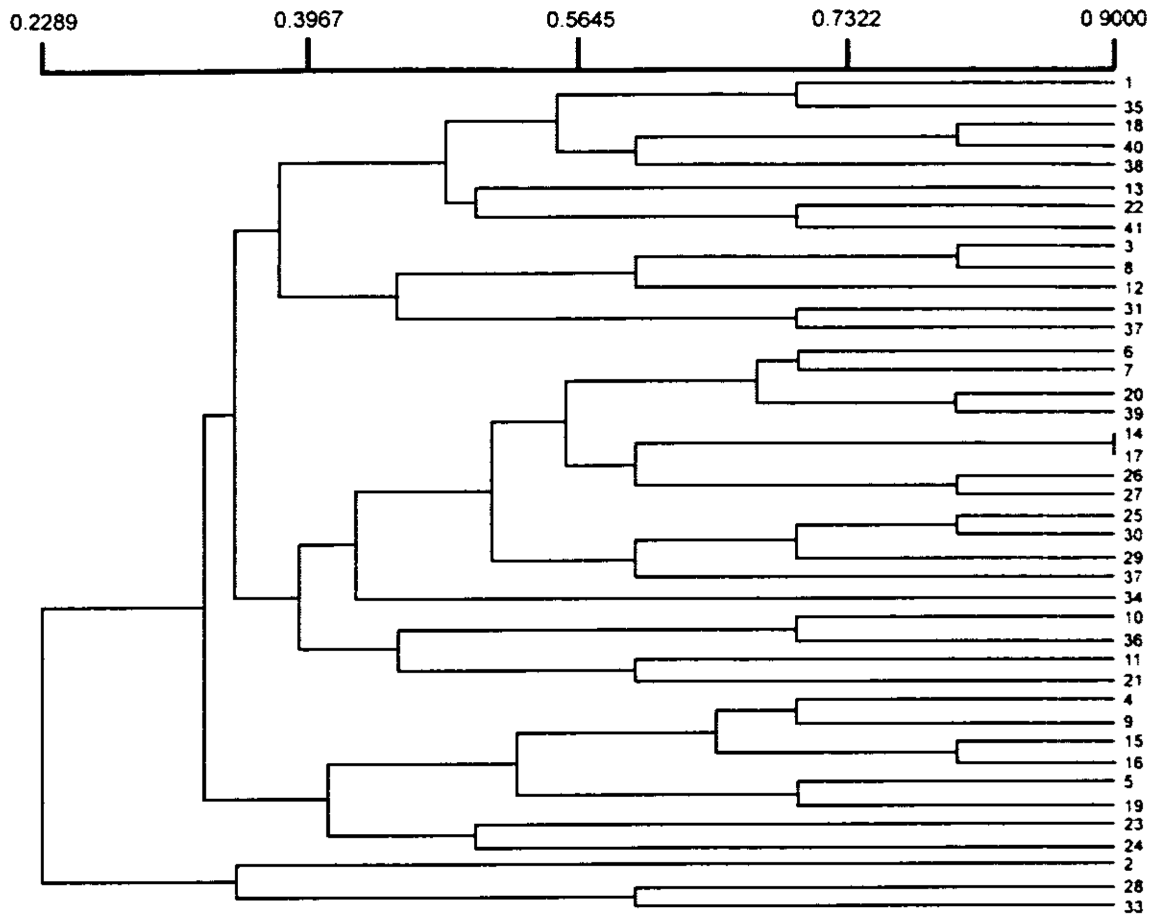

Figure 9. Clustering of 41 isolates of *B. sorokiniana* in a detached leaf inoculation test on 10 wheat genotypes given in Fig. 7.